

Construction of Pbs.PGK.PCR1

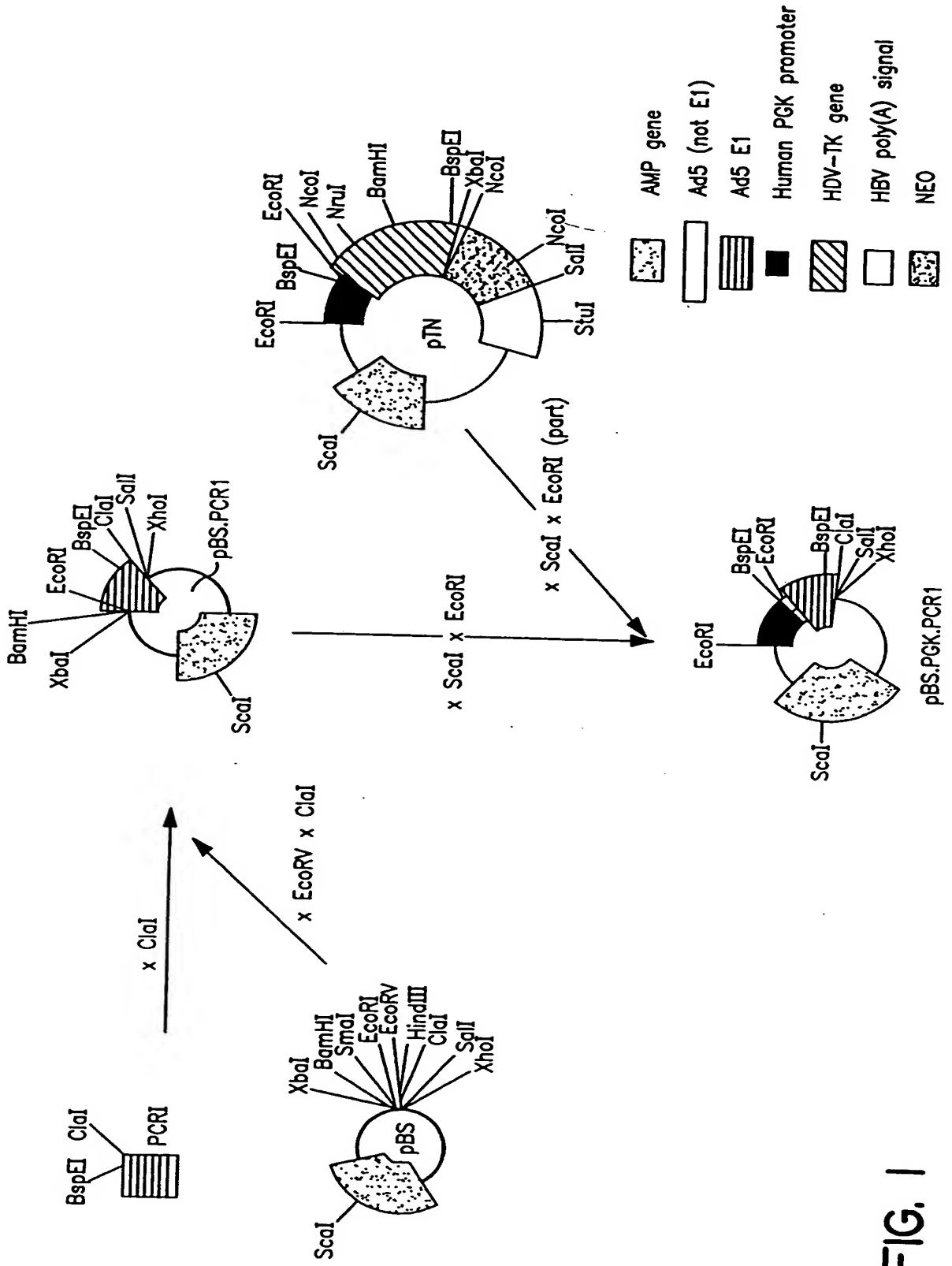


FIG. 1

Construction of pIG.E1a.E1b.X

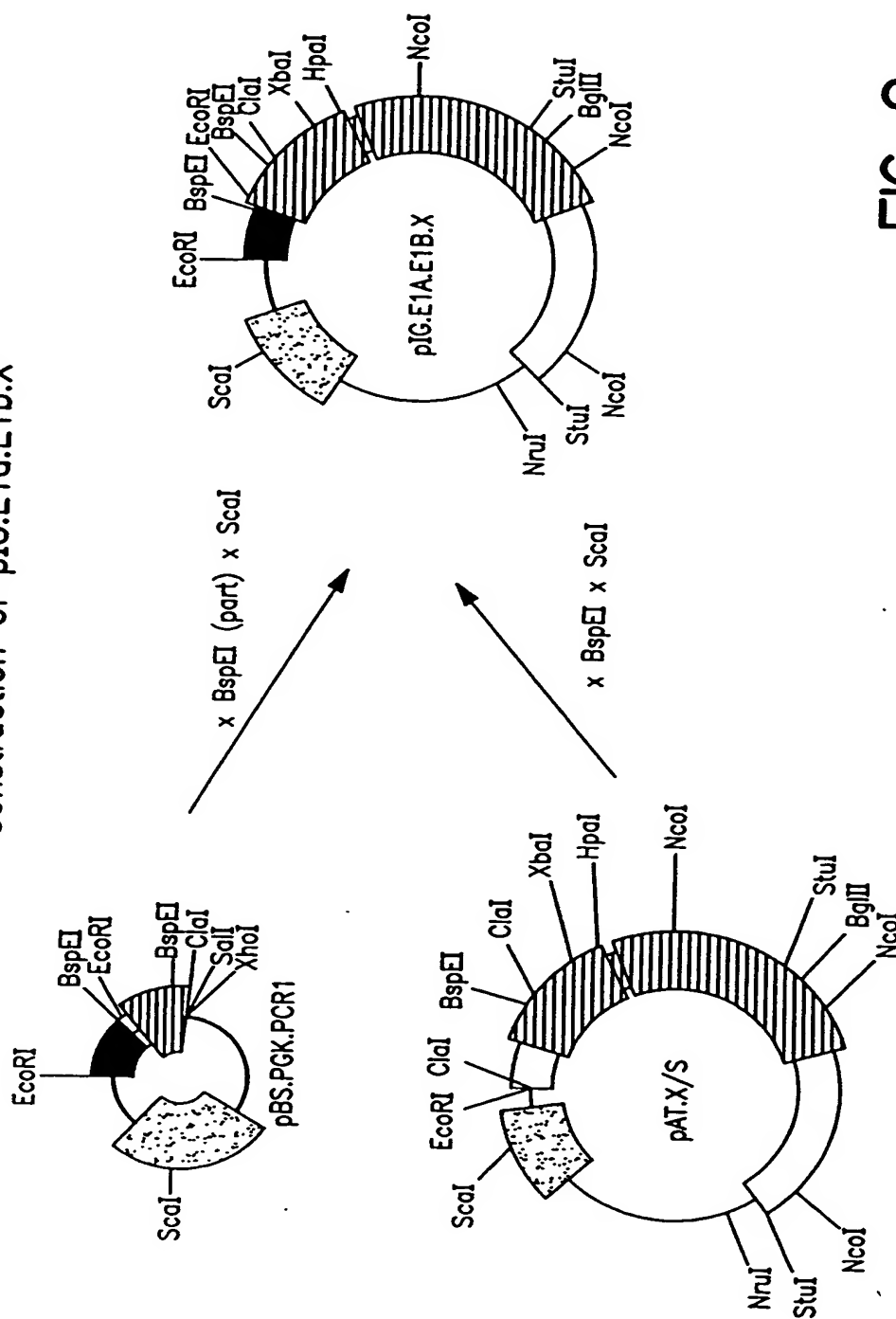


FIG. 2

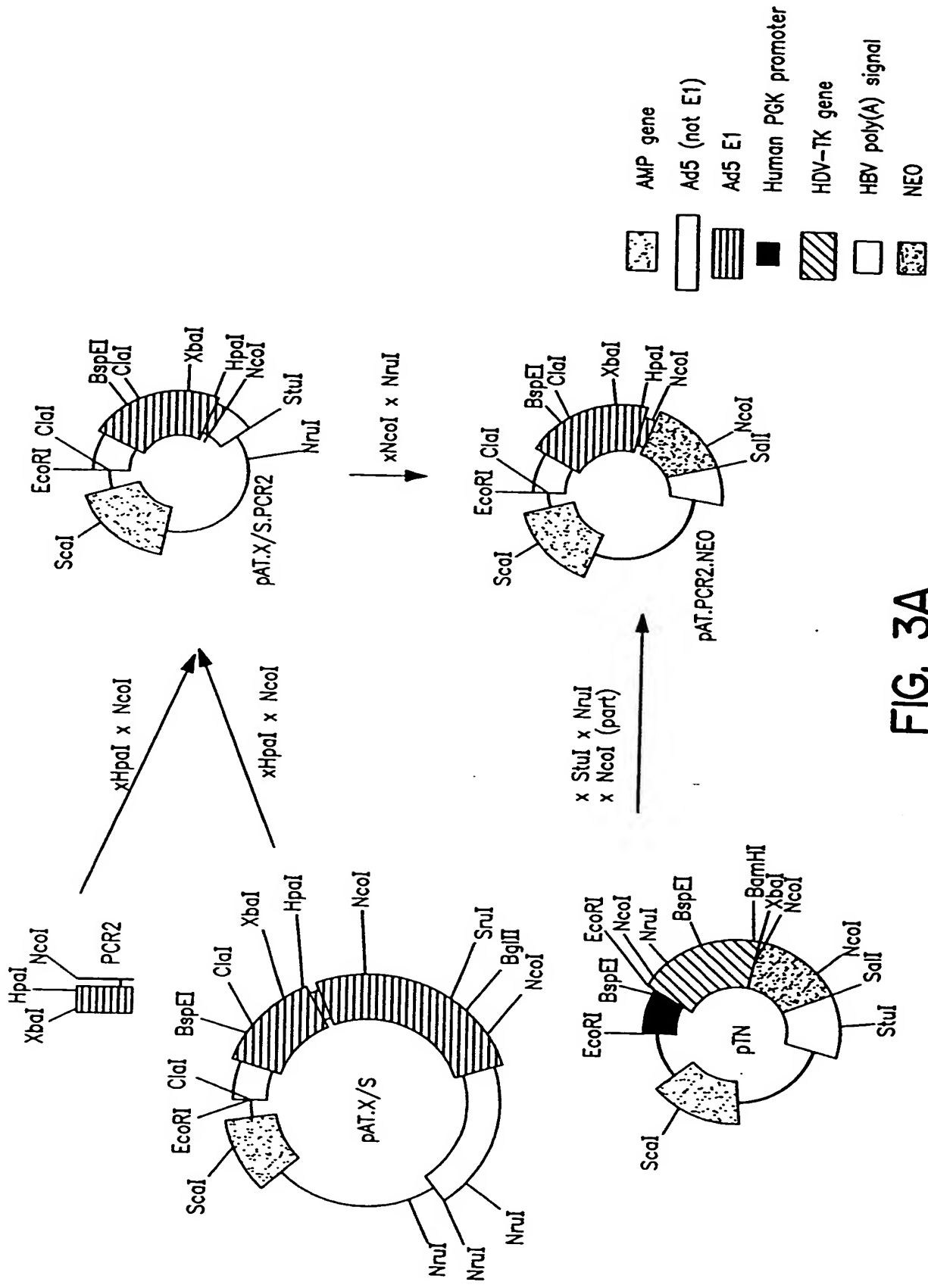


FIG. 3A

Construction of pIG.E1a.NEO

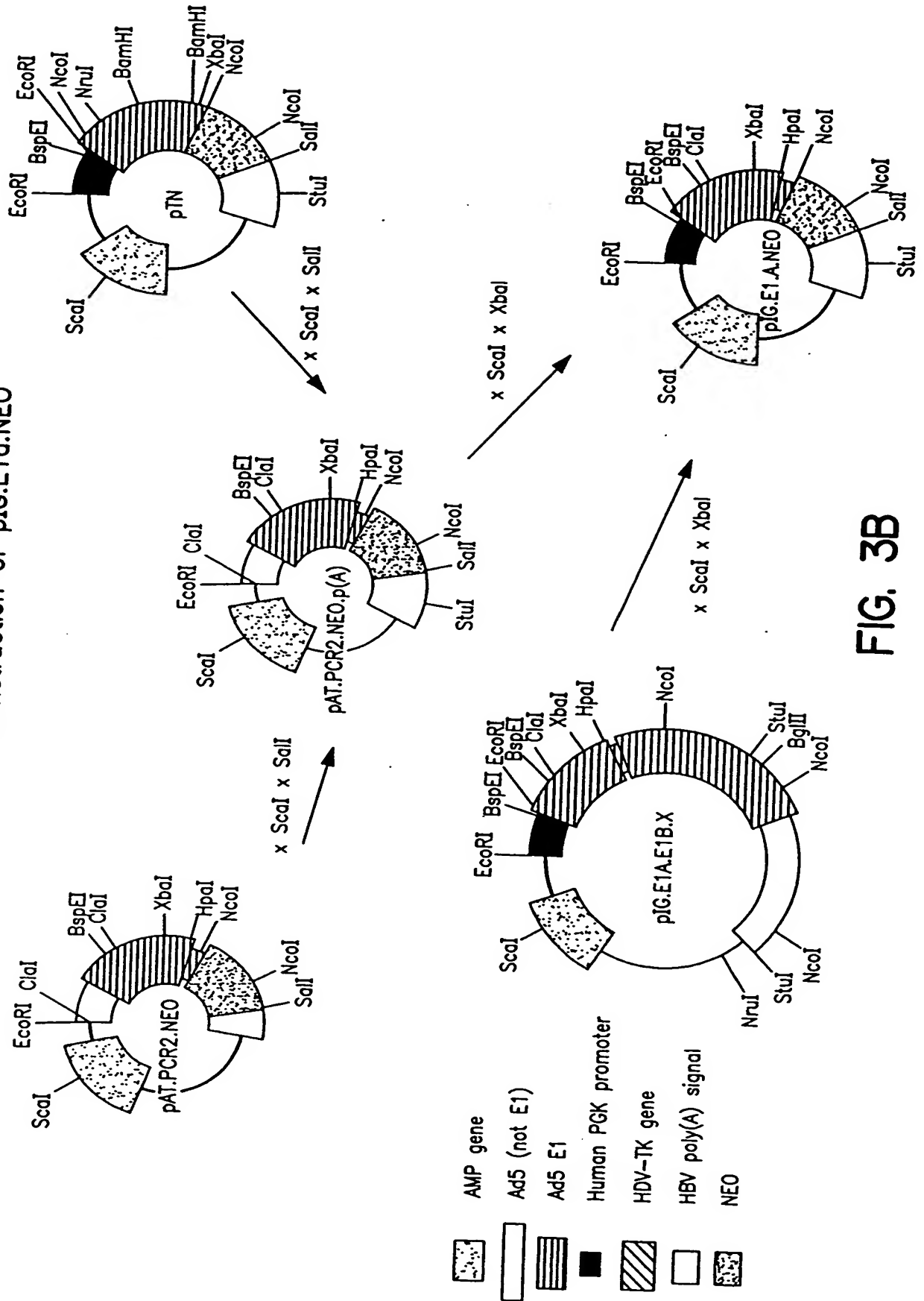
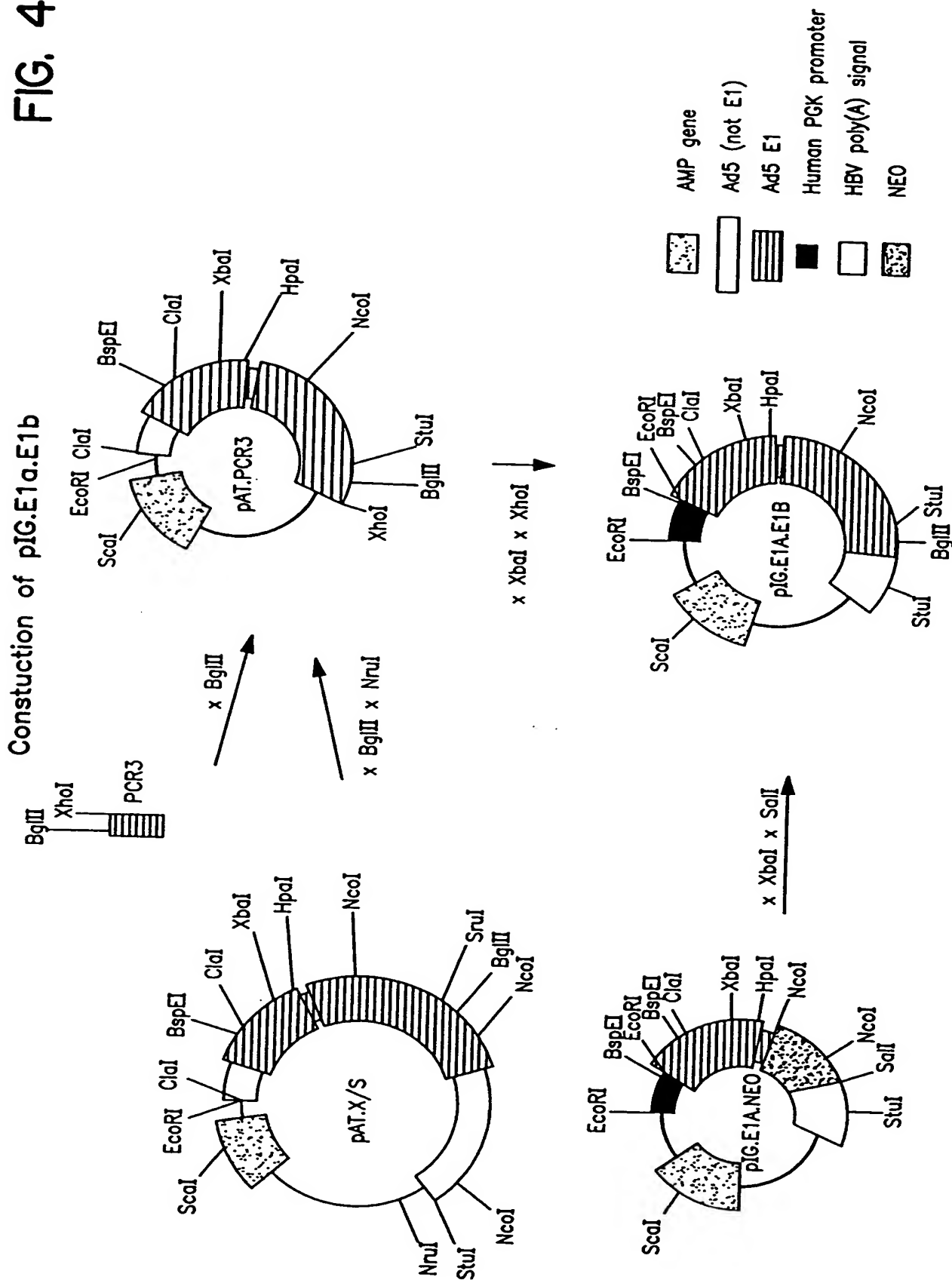


FIG. 3B

FIG. 4



Construction of pIG.NEO

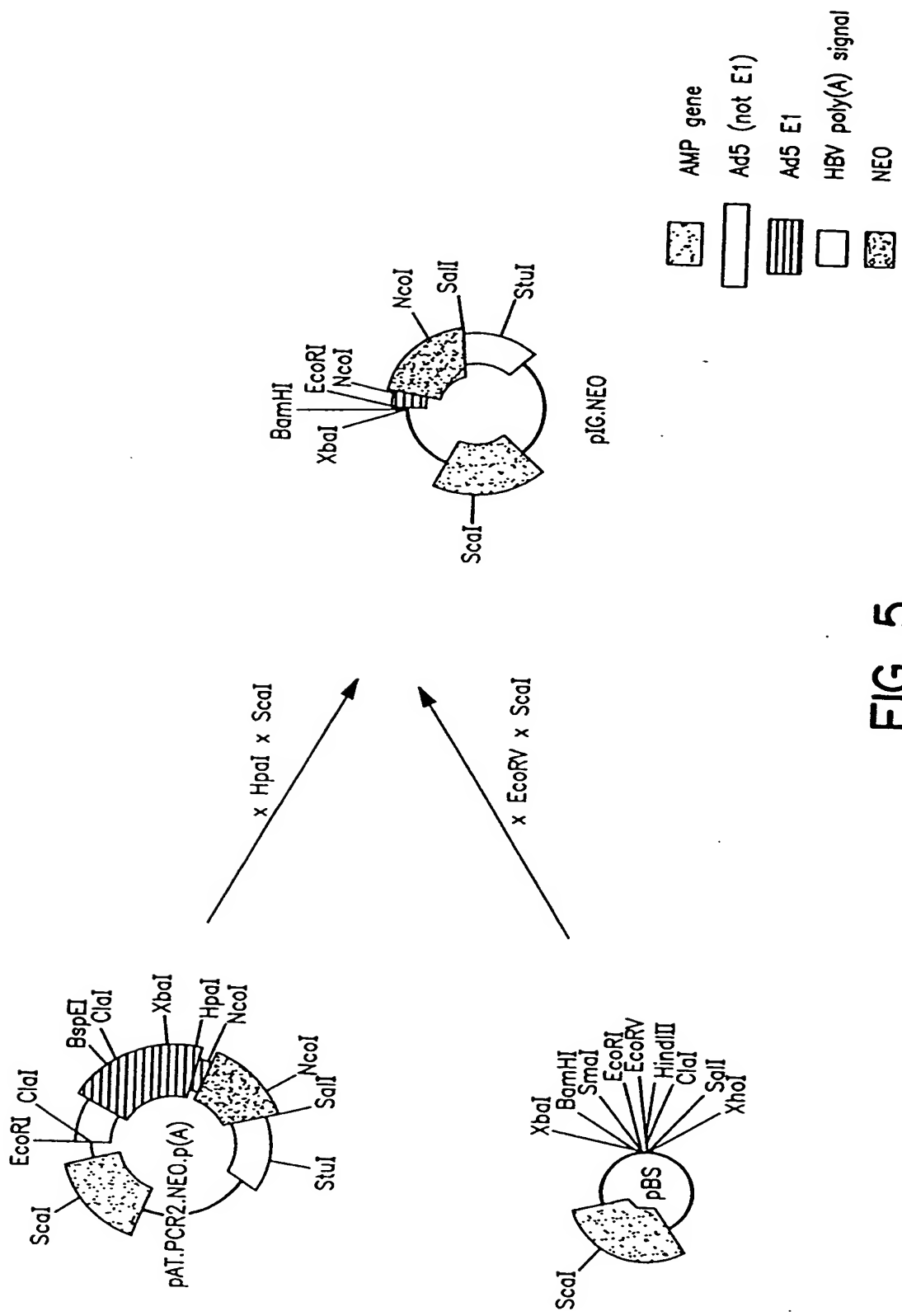
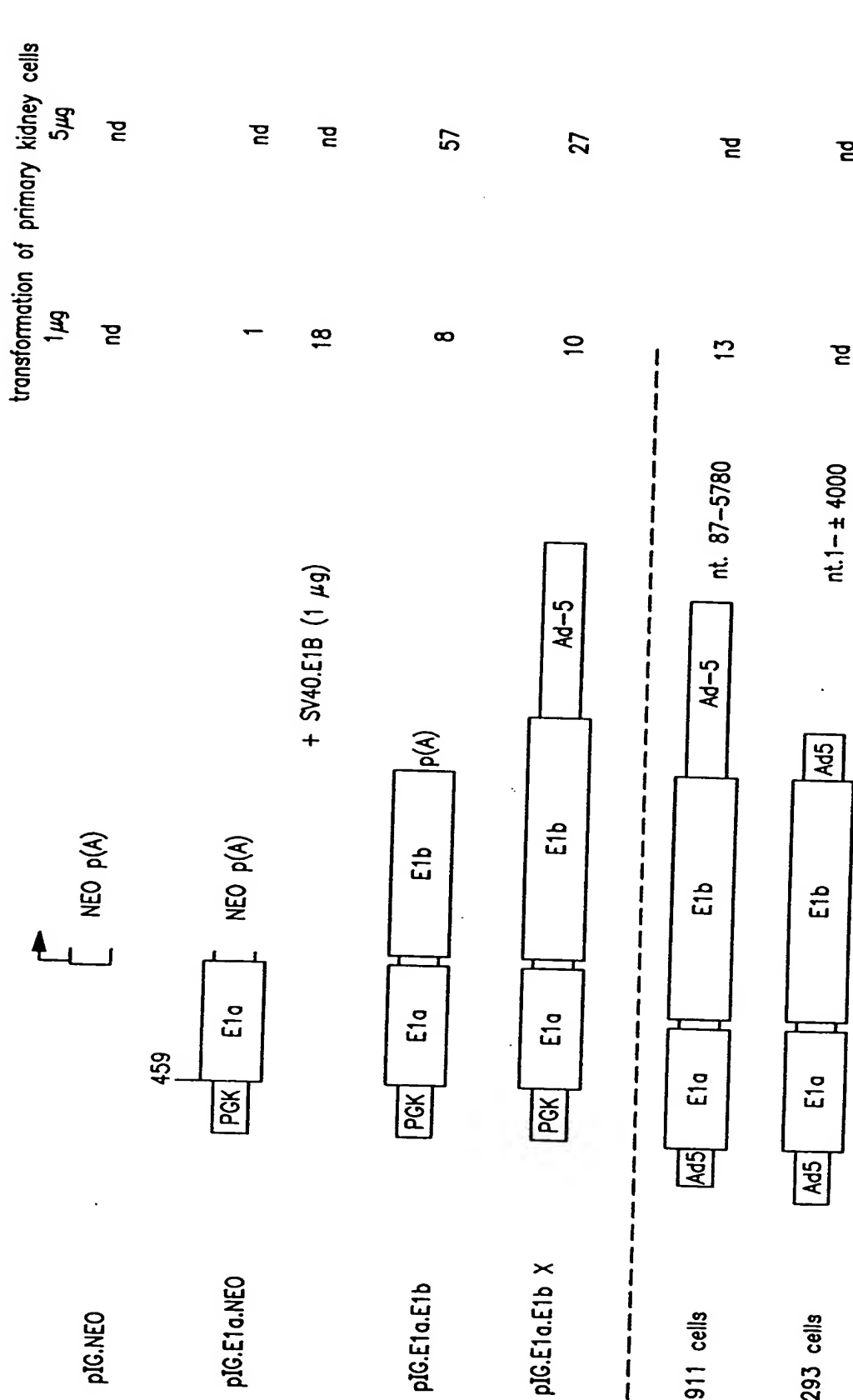


FIG. 5

Overview of available adenovirus packaging constructs and assessment of their capacity to transform primary kidney cells



*average of 5 plates 21 days after transection

FIG. 6

Western blotting analysis of A549 clones transfected with pIG.E1A.NEO and PER clones (HER cells transfected with pIG.E1A.E1B)

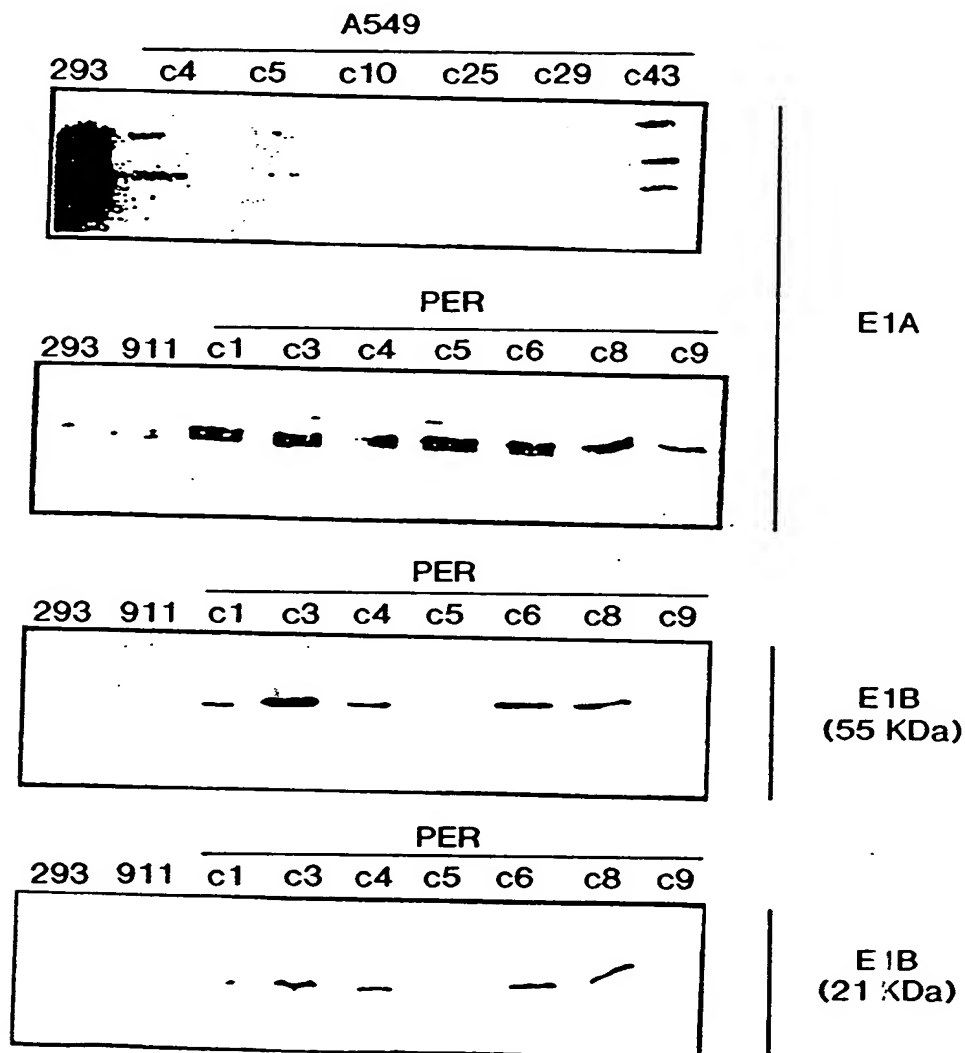


FIG. 7

Southern blot analyses of 293, 911 and PER cell lines

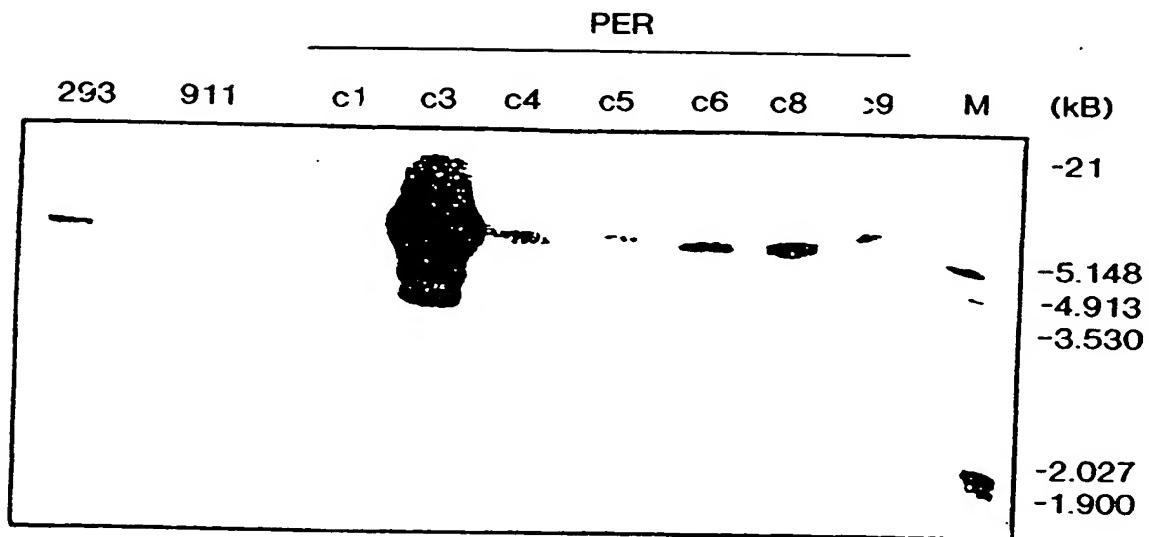


FIG. 8

Transfection efficiency of PER.C3, PER.C5, PER.C6 and 911 cells. Cells were cultured in 6-well plates and transfected (n=2) with 5 μ g pRSV.lacZ by calcium-phosphate co-precipitation. Forty-eight hours later the cells were stained with X-GAL. The mean percentage of blue cells is shown.

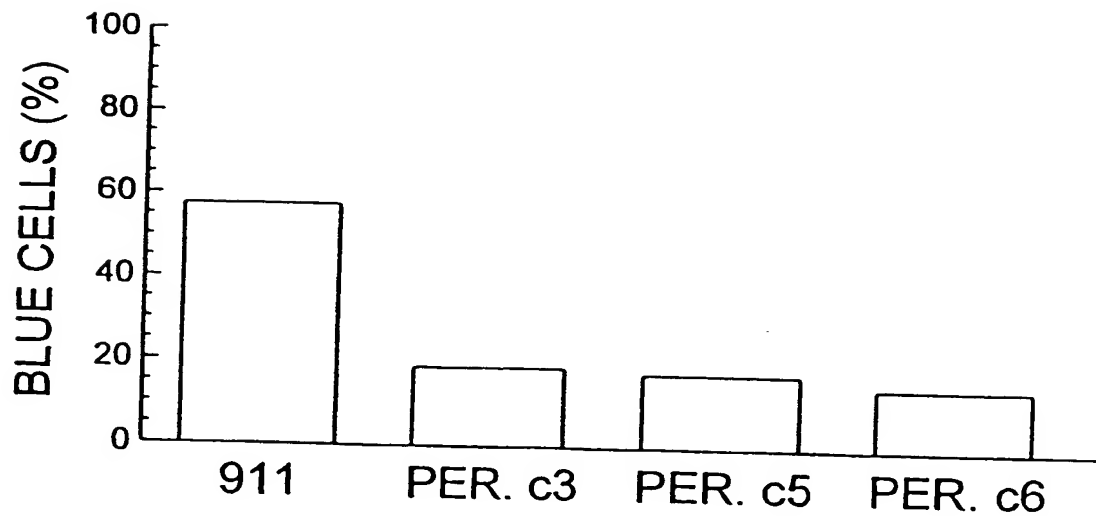


FIG. 9

Construction of pMLP1.TK

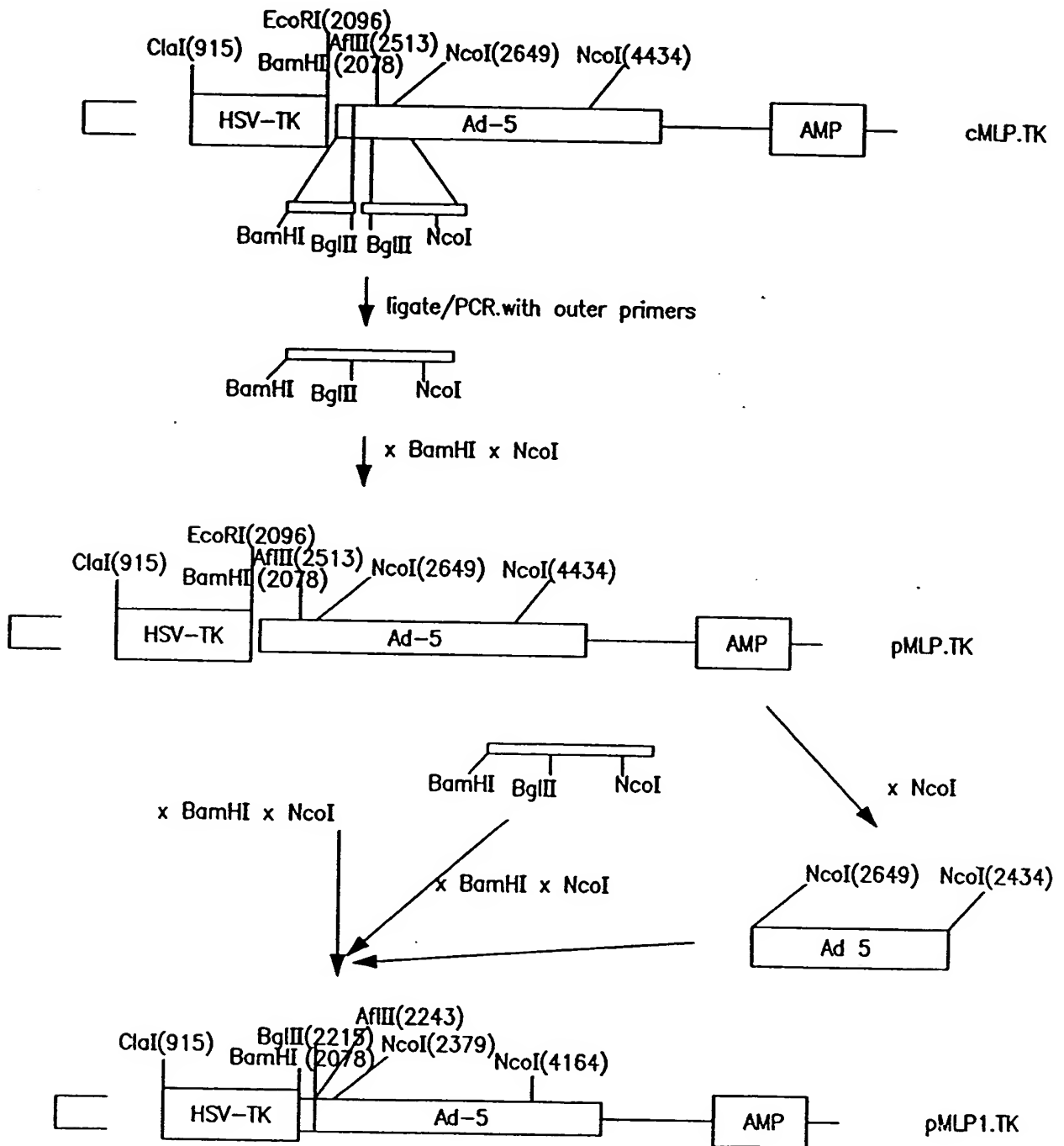


FIG. 10

New recombinant adenoviruses and packaging constructs without sequence overlap

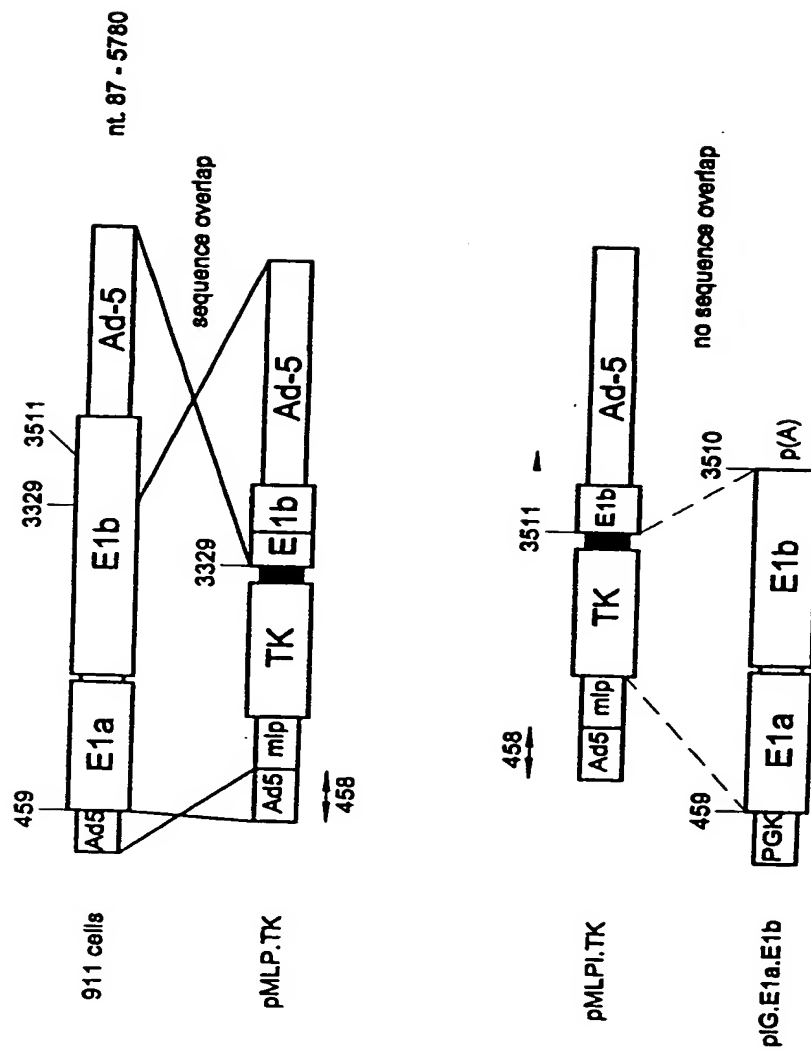
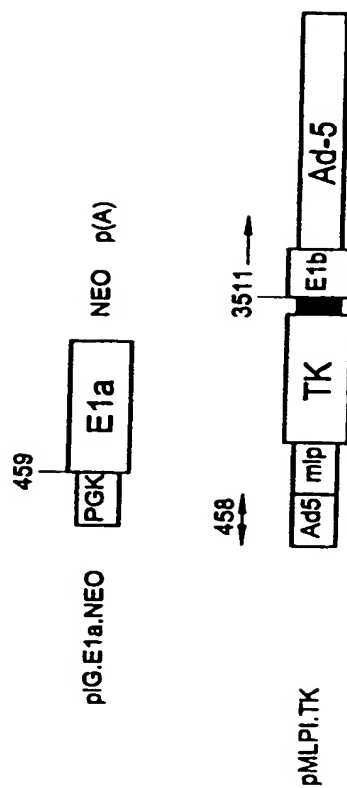


FIG. 1IA

Packaging system based on primary cells

New recombinant adenoviruses and packaging constructs without sequence overlap



Packaging system based on established cell lines: transfection with E1a and selection with G418 **FIG. 1B**

10074668.070002

Generation of recombinant adenovirus

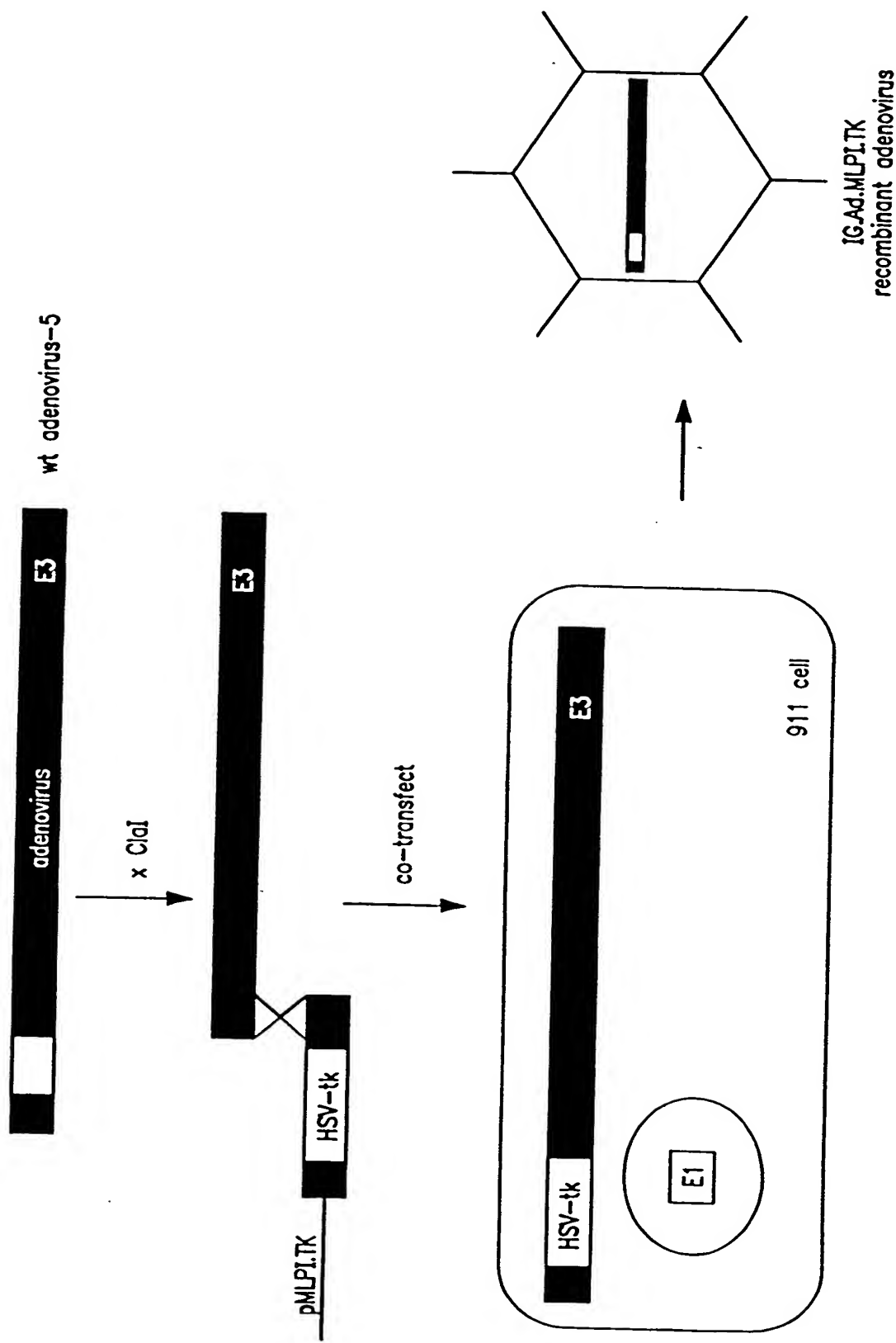


FIG. 12

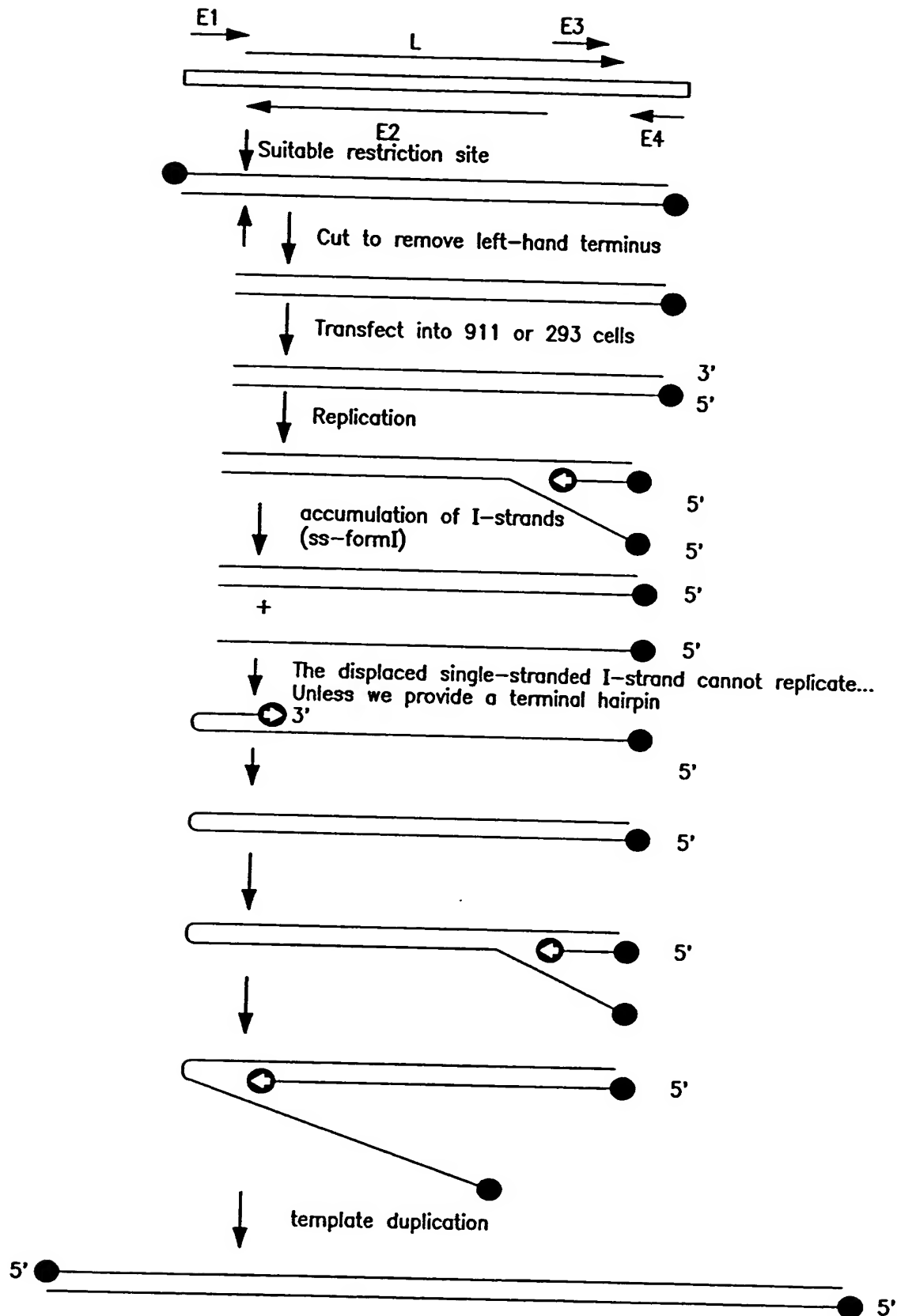


FIG. 13

Replication of Adenovirus

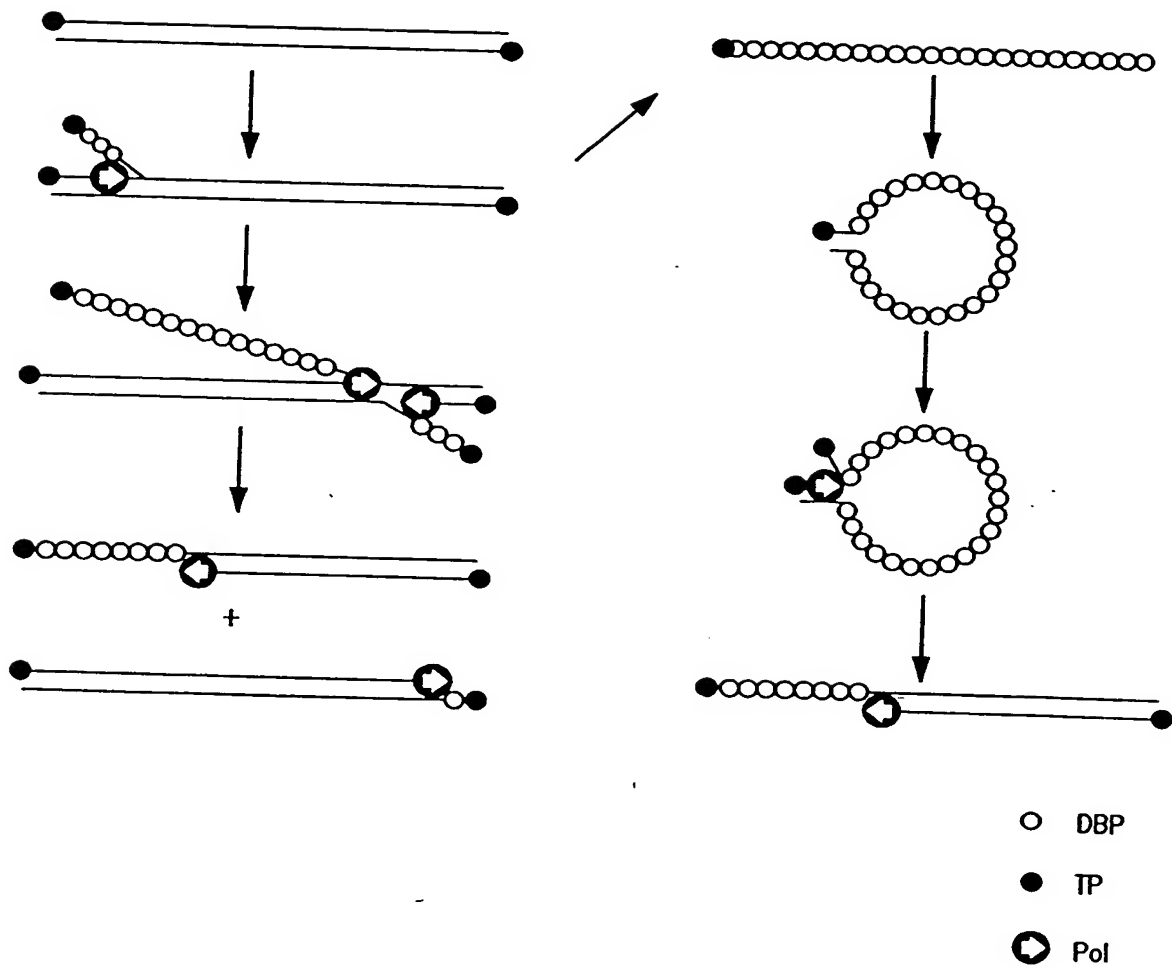


FIG. 14

The potential hairpin conformation of a single-stranded DNA molecule that contains the HP/asp sequences used in these studies. Restriction with the restriction endonucleases *Asp718I* of plasmid pICLHa, containing the annealed oligonucleotide pair HP/asp1 en HP/asp2 will yield a linear double-stranded DNA fragment. In cells in which the required adenovirus genes are present, replication can initiate at the terminus that contains the ITR sequence. During the chain elongation, the one of the strands will be displaced. The terminus of the single-stranded displaced-strand molecule can adopt the conformation depicted above. In this conformation the free 3'-terminus can serve as a primer for the cellular and/or adenovirus DNA polymerase, resulting in conversion of the displaced strand in a double-stranded form.

```

5'-GTACACTGACCTAGTGCCGCCCGGGCA
      ||||| A
3'-GATCACGGCGGGCCCGA

```

FIG. 15

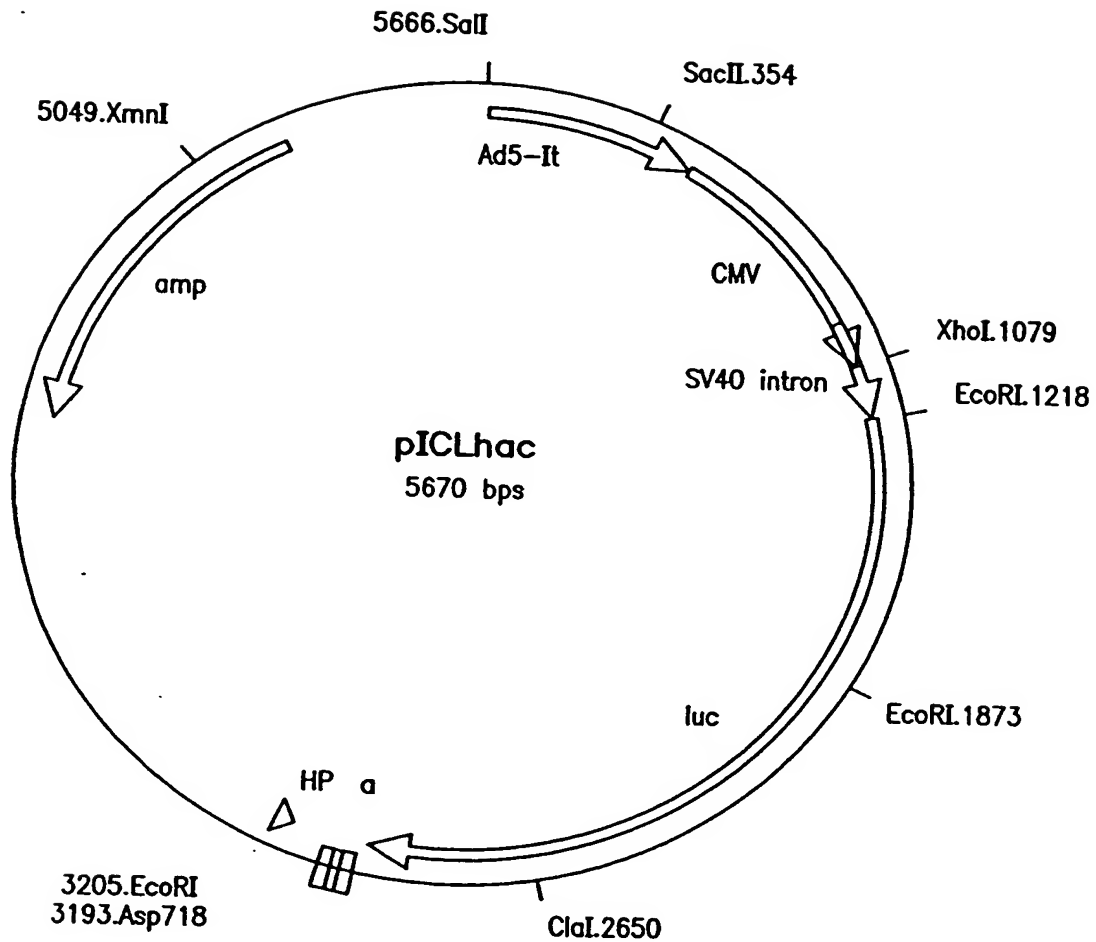


FIG. 16

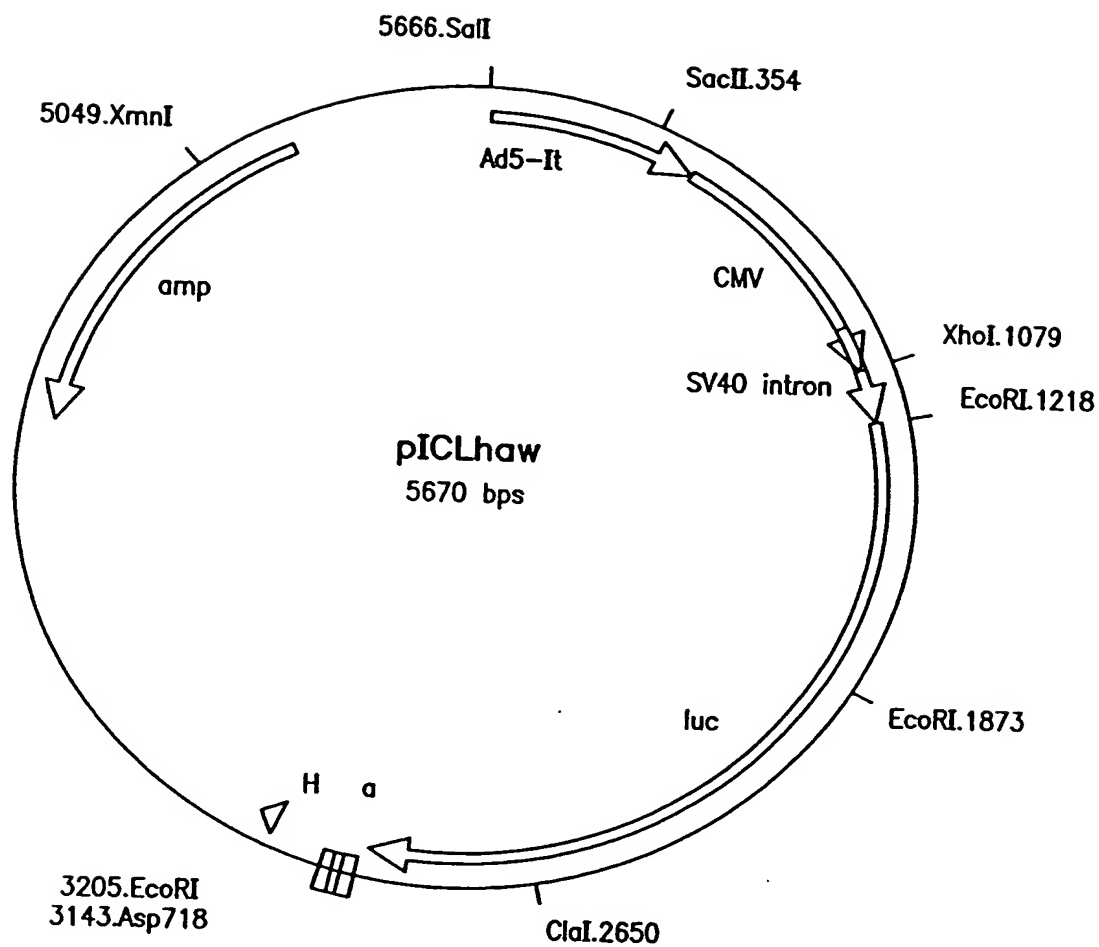


FIG. 17

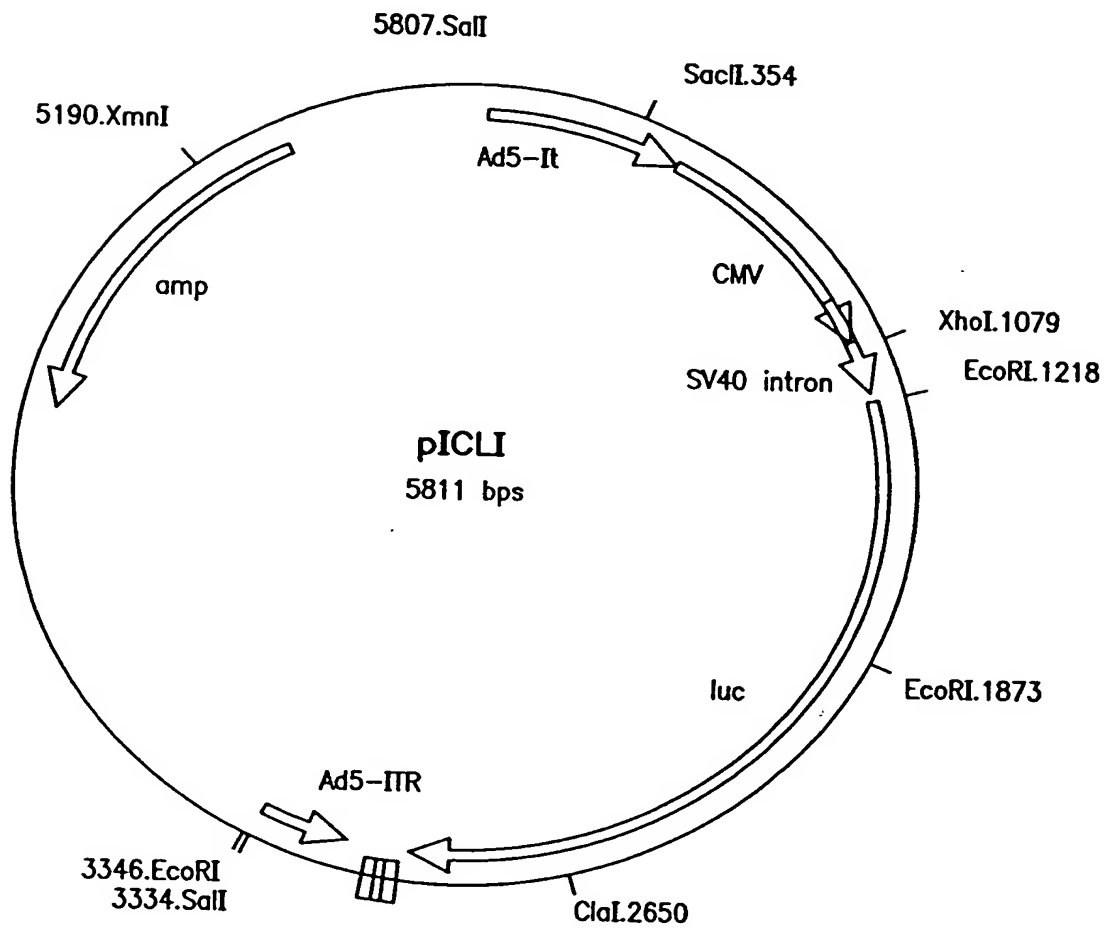


FIG. 18

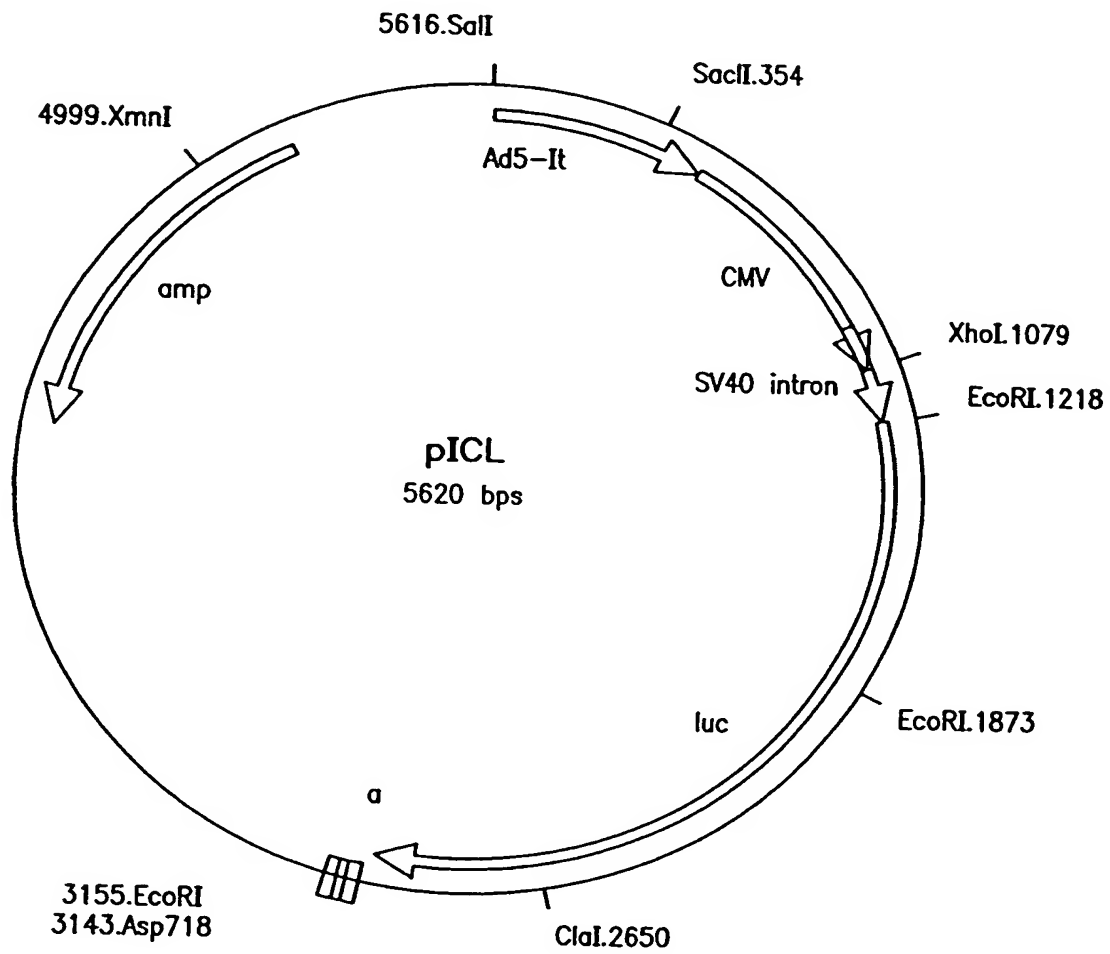


FIG. 19

Cloned adenovirous fragments

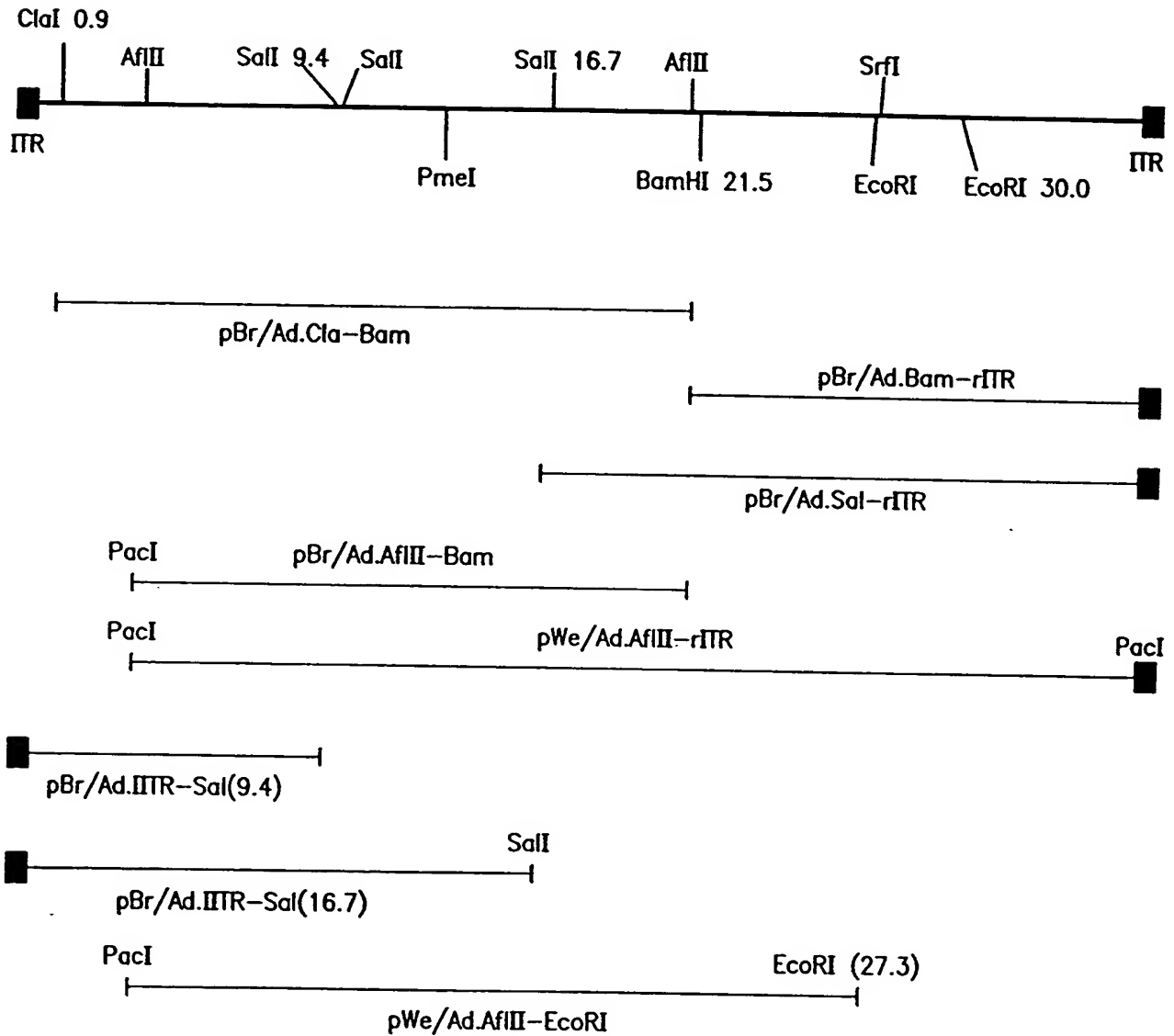


FIG. 20

Adapter plasmid pAd5/L420-HSA

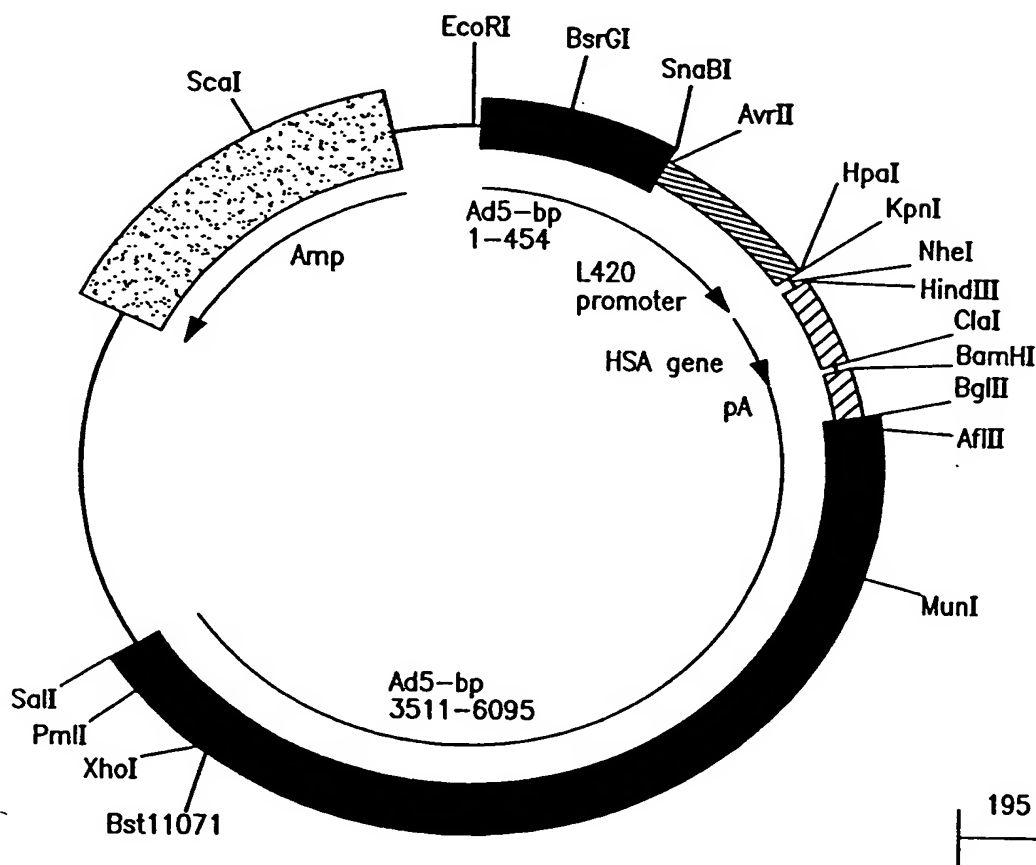


FIG. 21

Adapter plasmid pAd5/CLIP

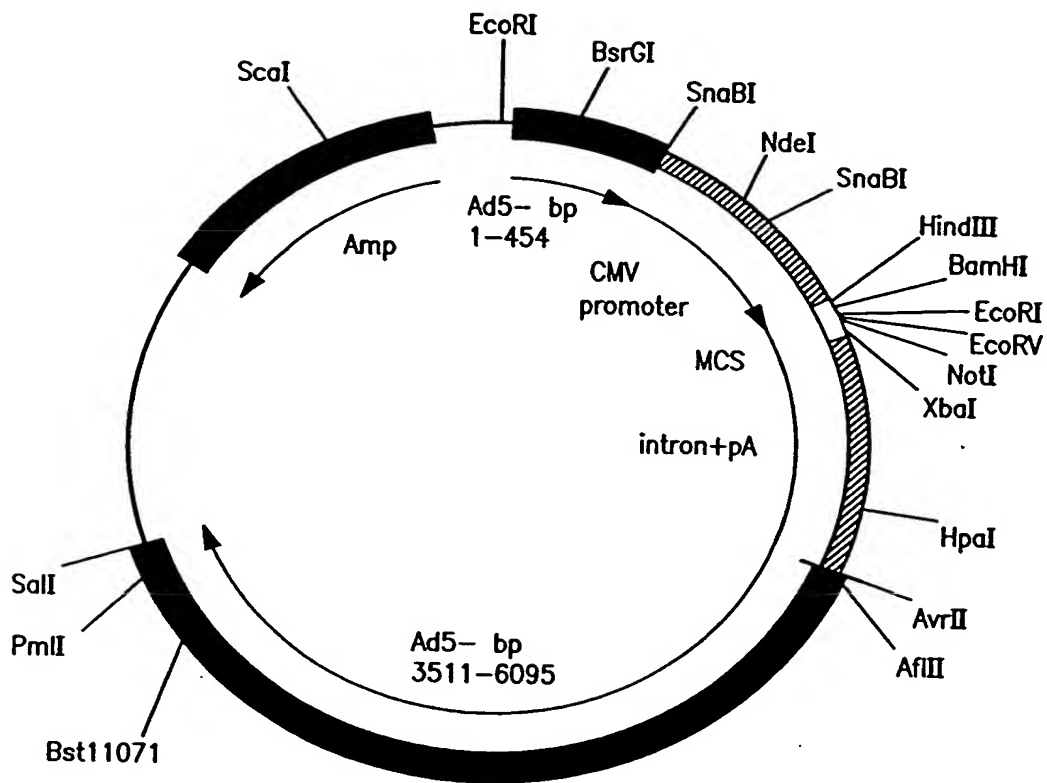


FIG. 22

Generation of recombinant adenoviruses

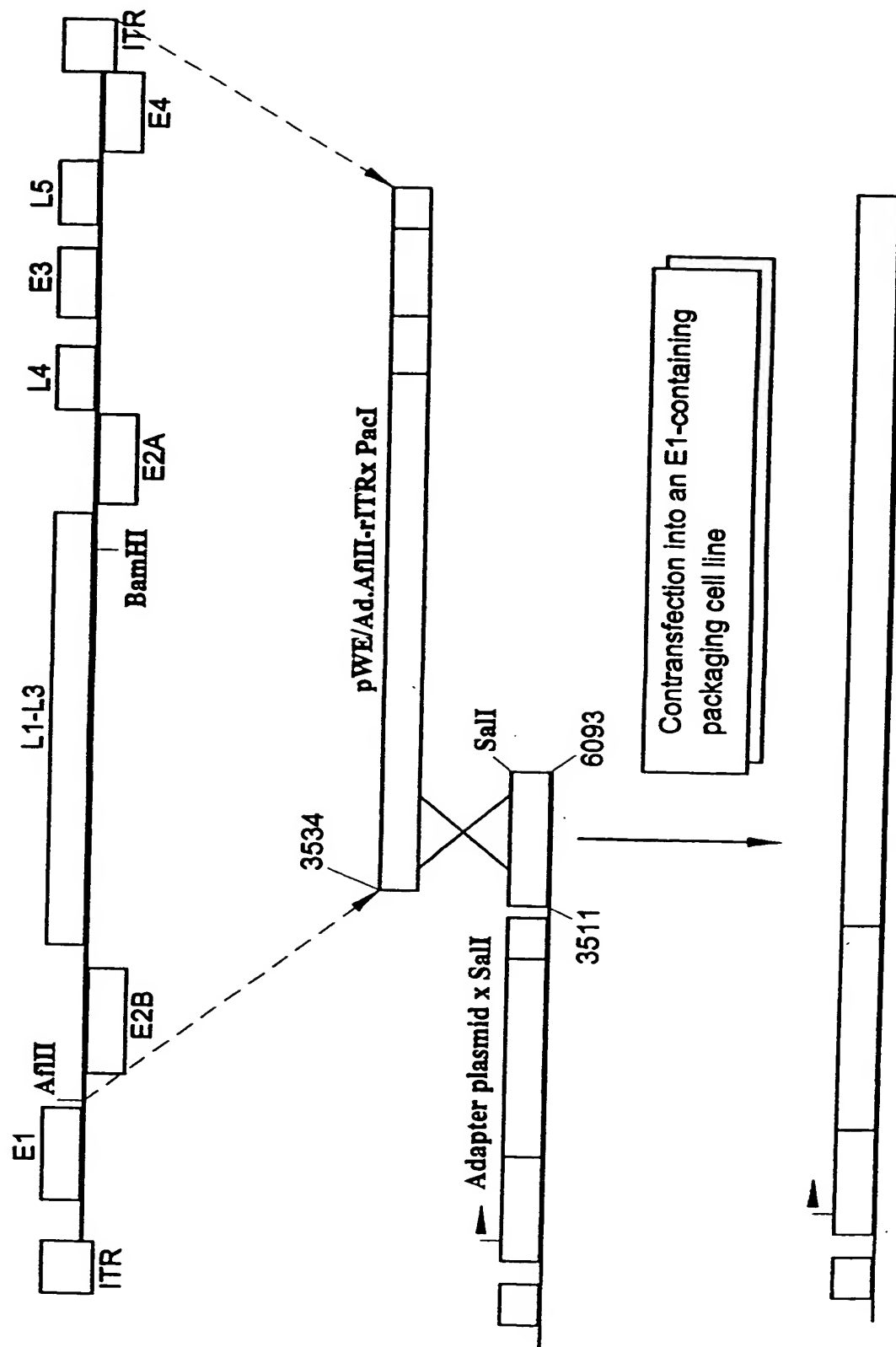


FIG. 23

Minimal adenovirus vector pMV/L420H

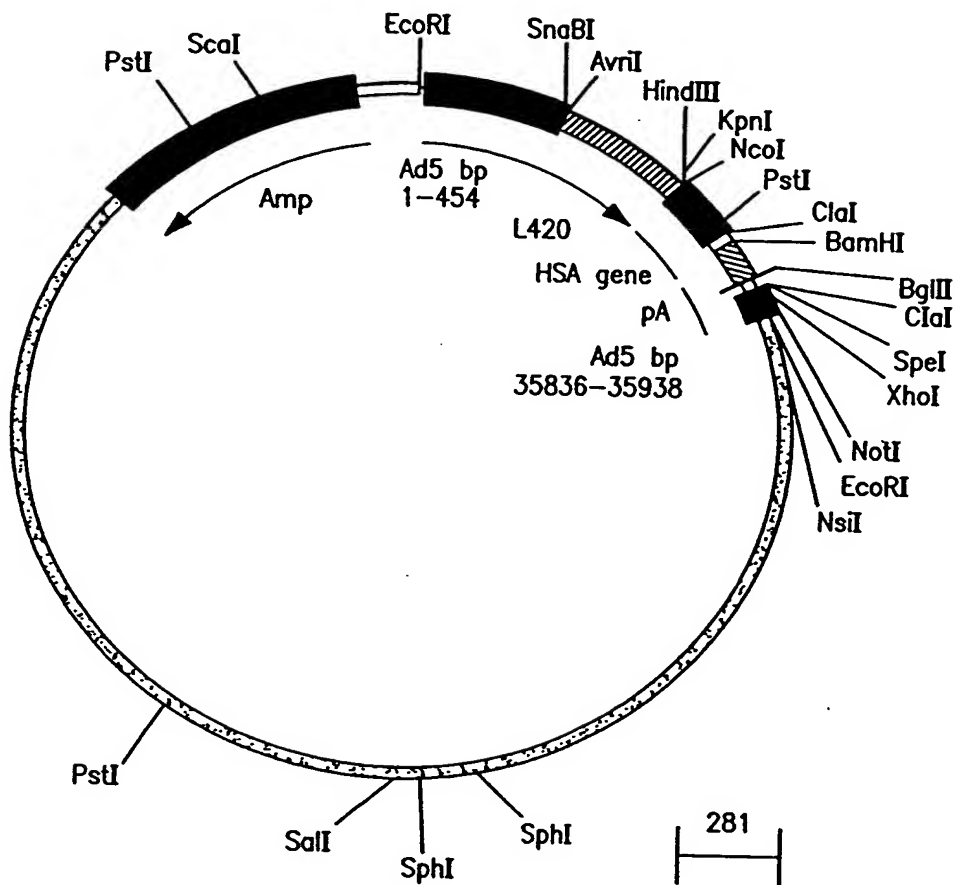


FIG. 24

Construction of pWE/AdΔ5'

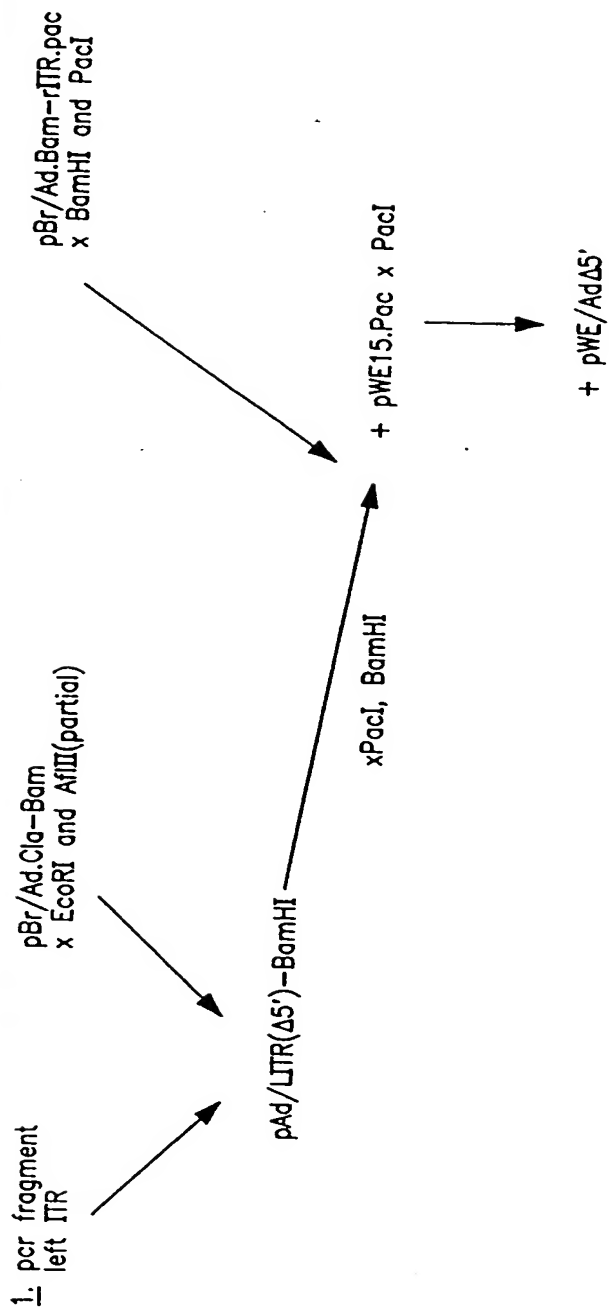
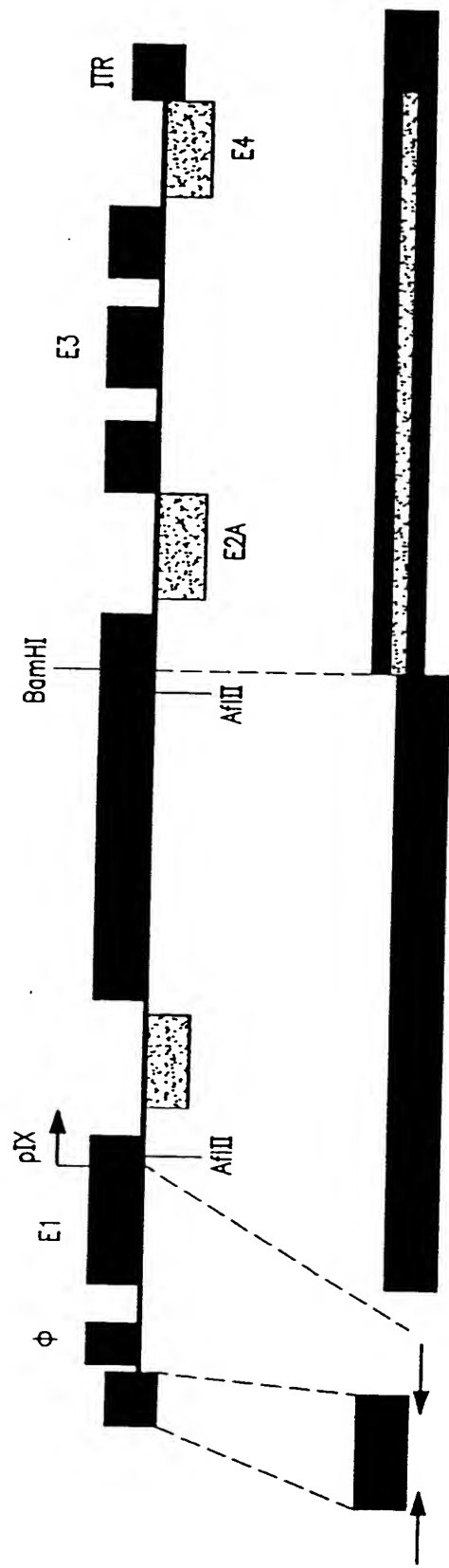


FIG. 25

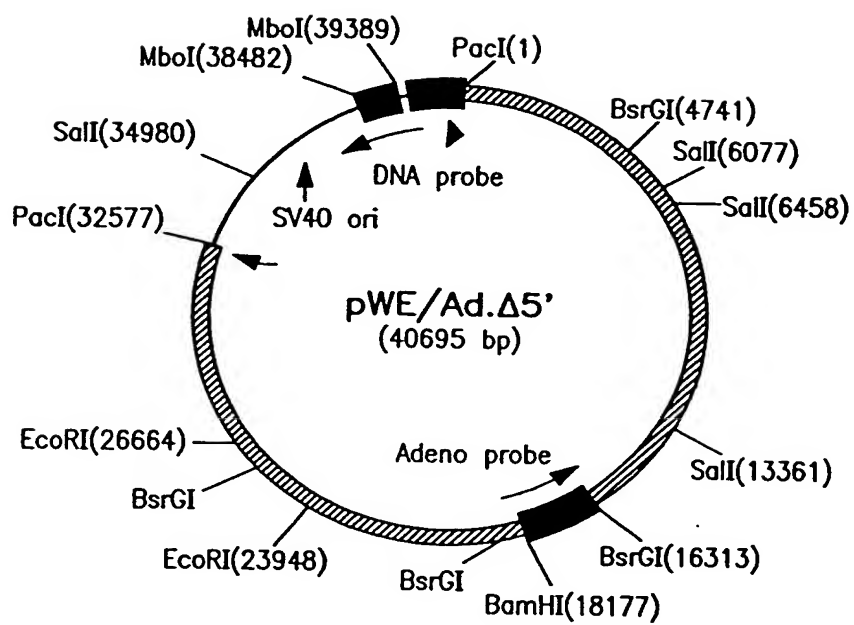


FIG. 26A

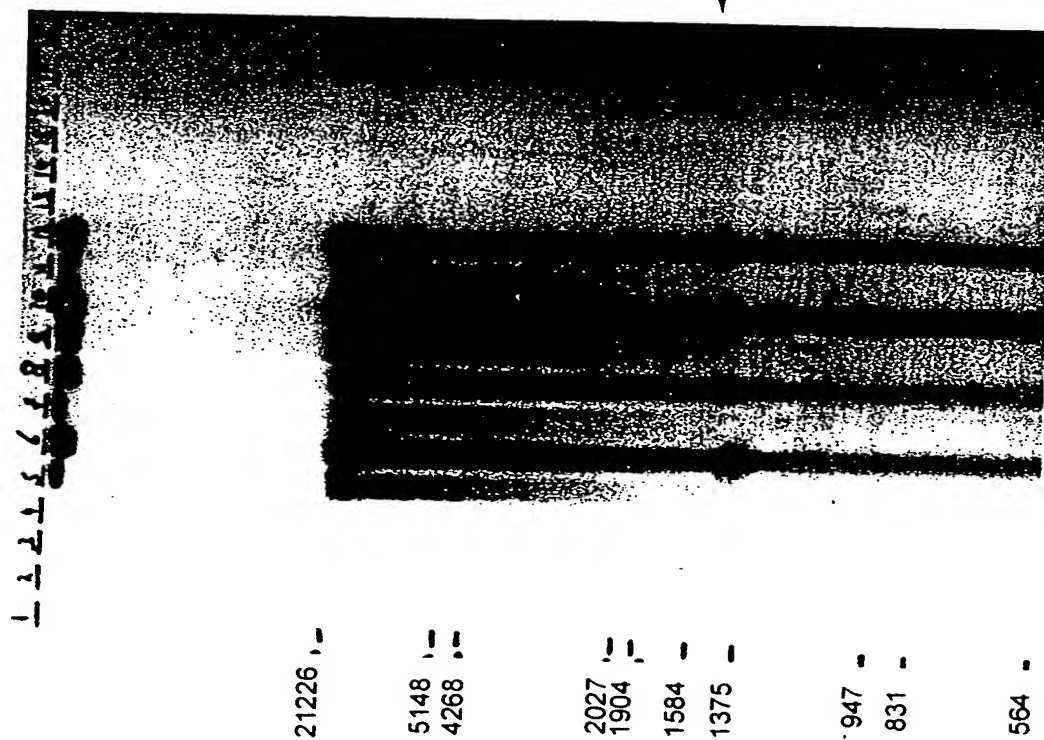


FIG. 26B

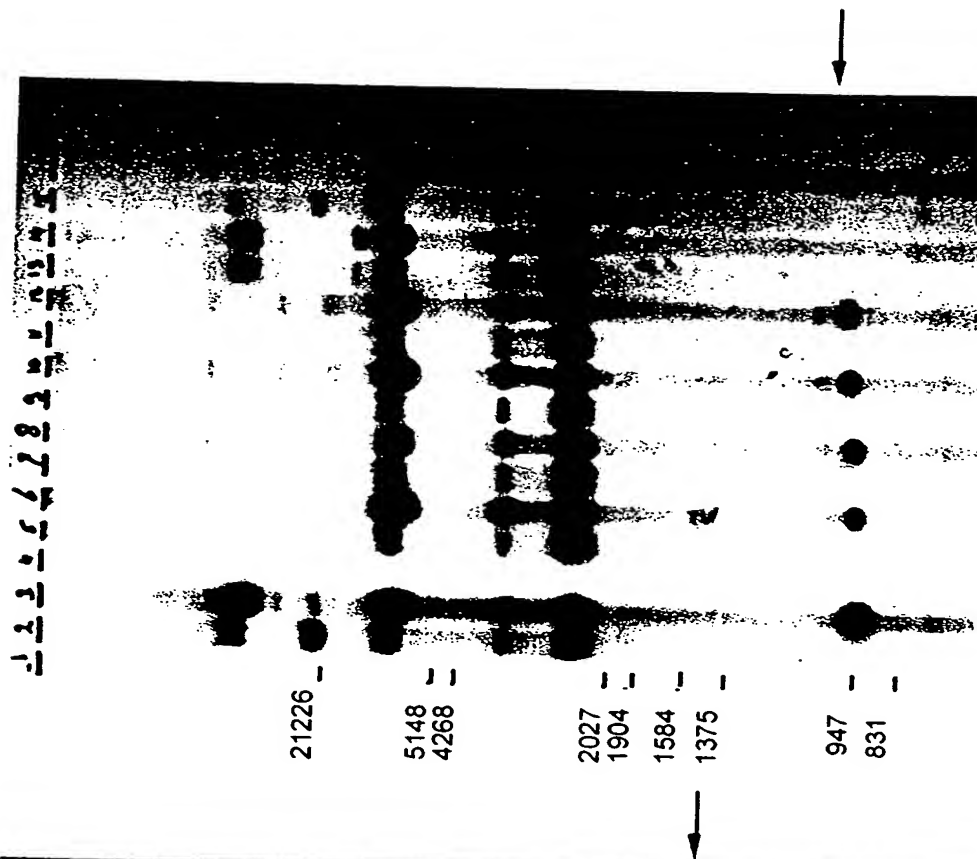
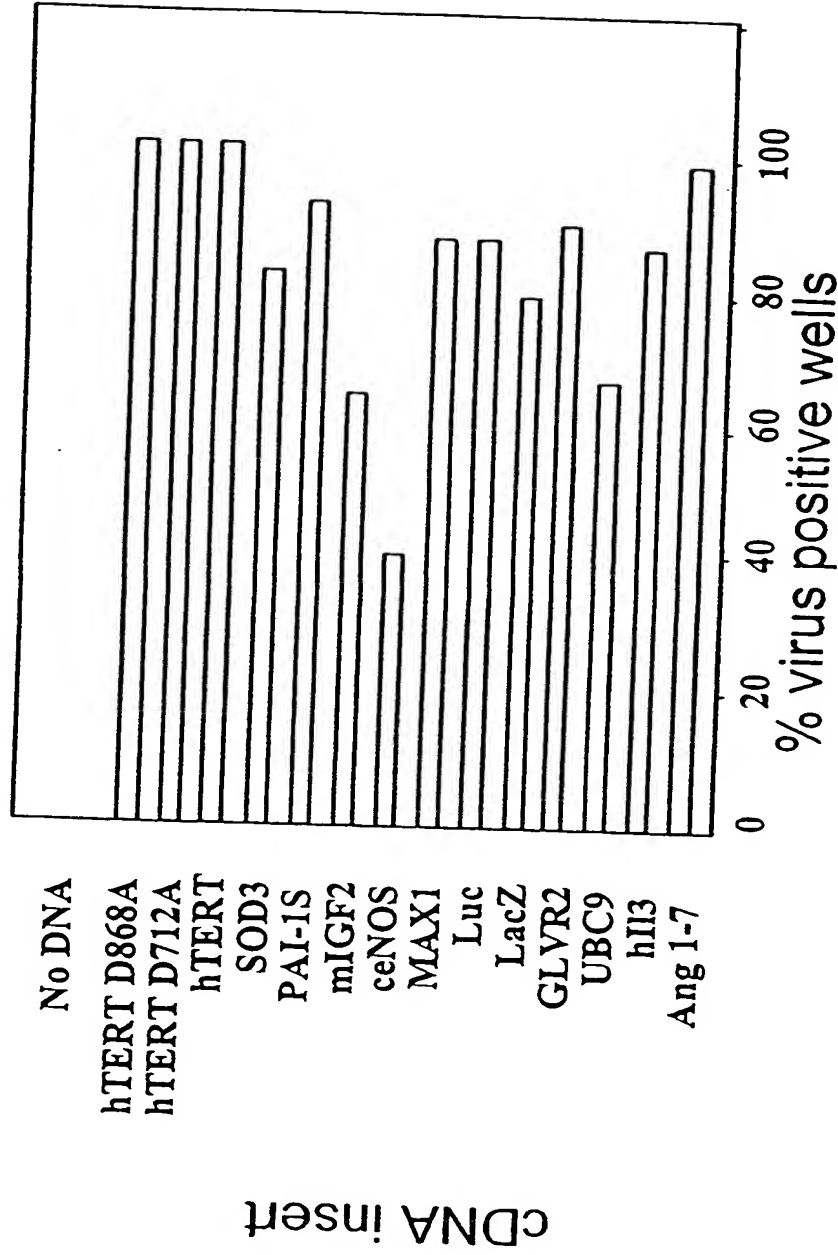


FIG. 26C



Average percentage CPE efficiency: 86 %

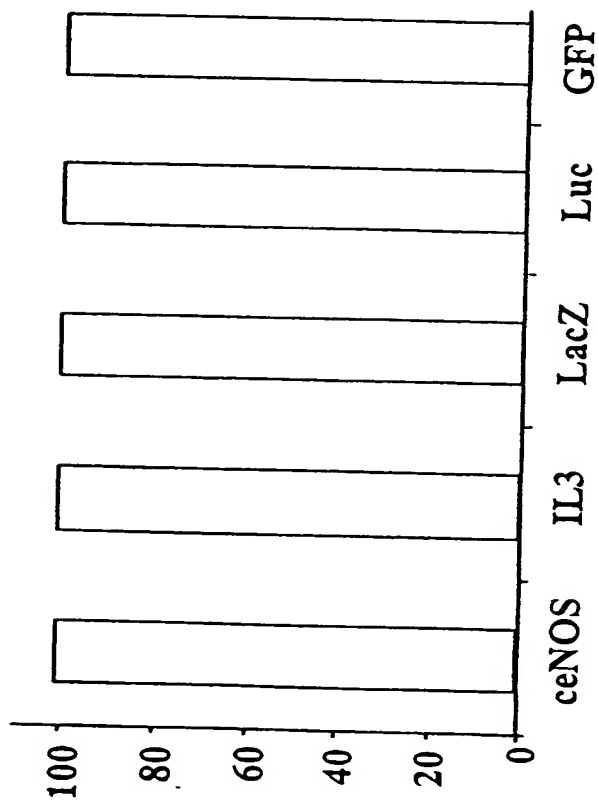
FIG. 27

Gene Insert kb

• ceNOS	3.6	Average titer 0.8 ± 0.7 x 10 ⁹ pfu/ml
• hTERT	3.5	
• hTERT D712A	3.5	
• lacZ	3.2	
• hCAT1	2.2	
• GLVR2	2.0	
• Luc	1.7	
• SOD3	1.4	
• MAX1	.550	
• hVEGF121	.511	
• hIL3	.434	
• UBC9	.412	
• ANG1-7	.104	

FIG. 28

% wells producing functional virus



Gene	Number of CPE+ wells
ceNOS	19/19
IL3	7/7
lacZ	36/36
Luc	40/40
GFP	48/48

Gene	Number of plaques
ceNOS	9/9
IL3	9/9
lacZ	40/40
Luc	9/9
EGFP	IP
GLVR2	9/9

FIG. 29

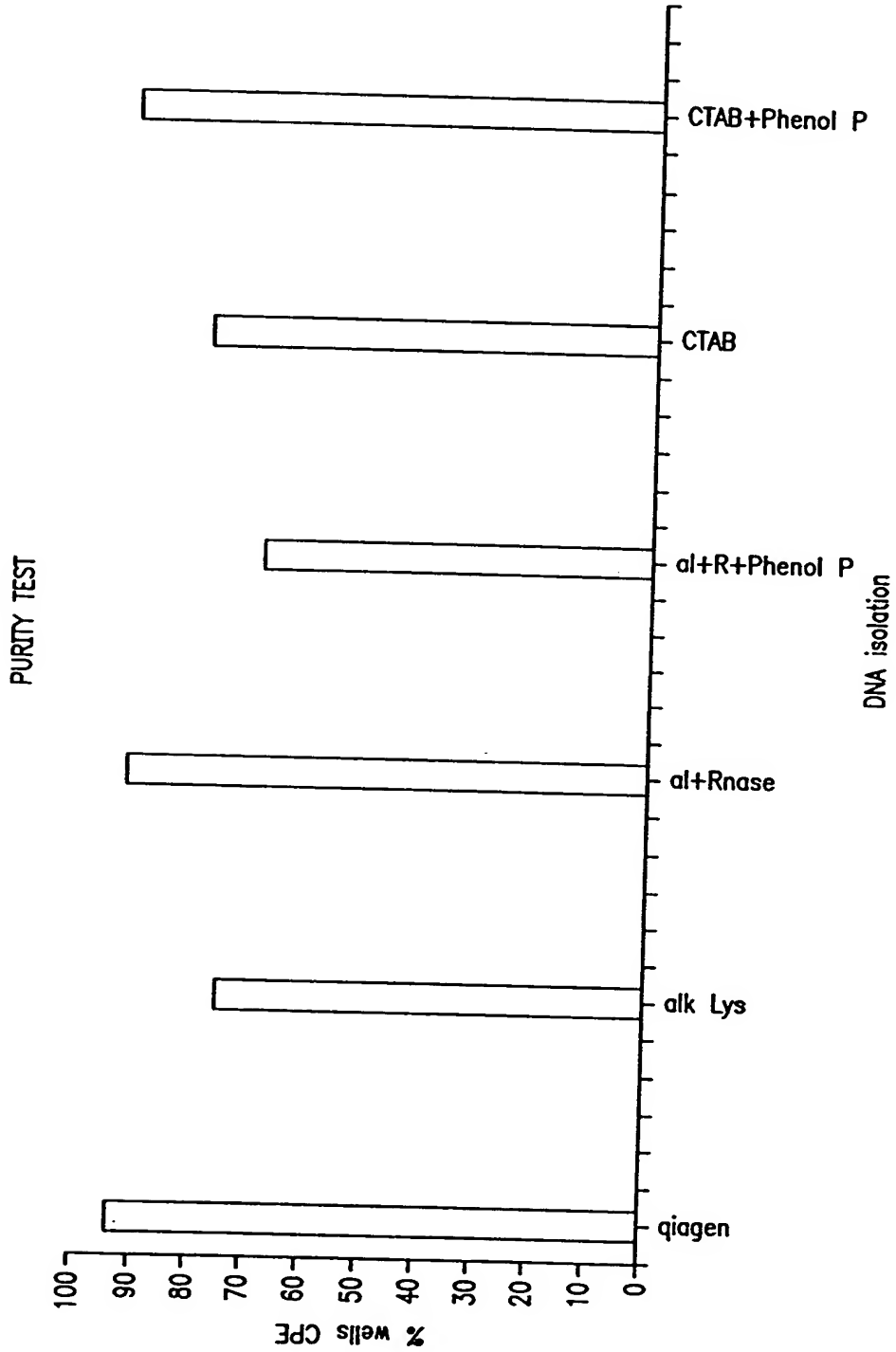


FIG. 30

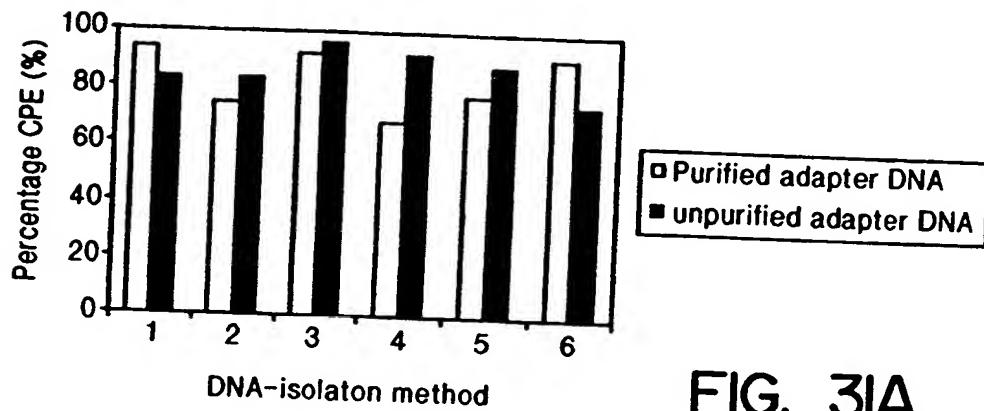


FIG. 3IA

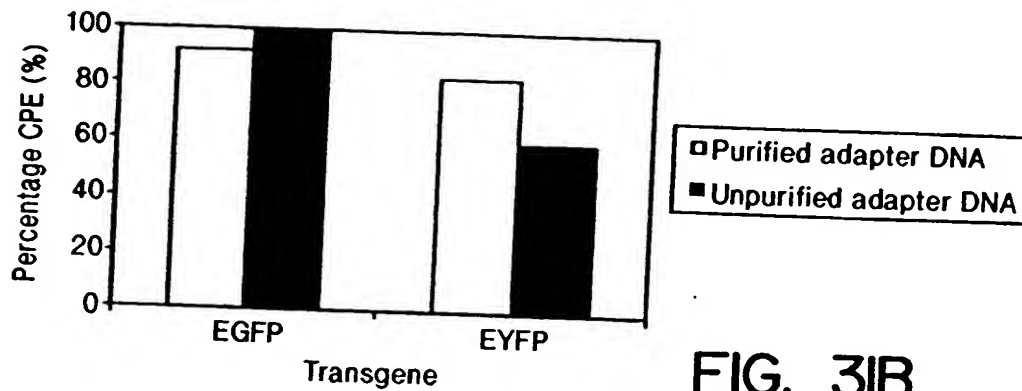


FIG. 3IB

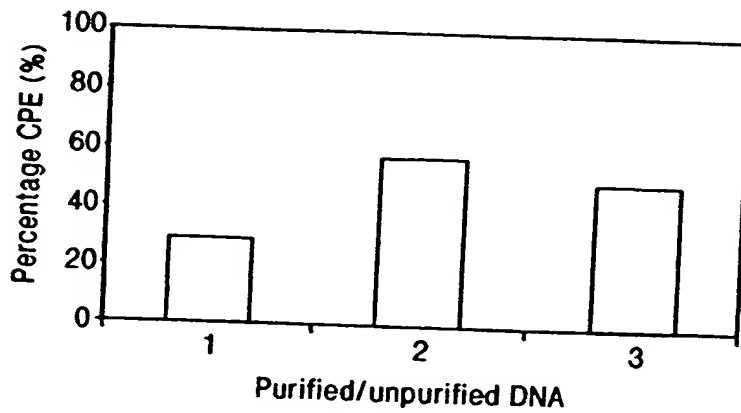


FIG. 3IC

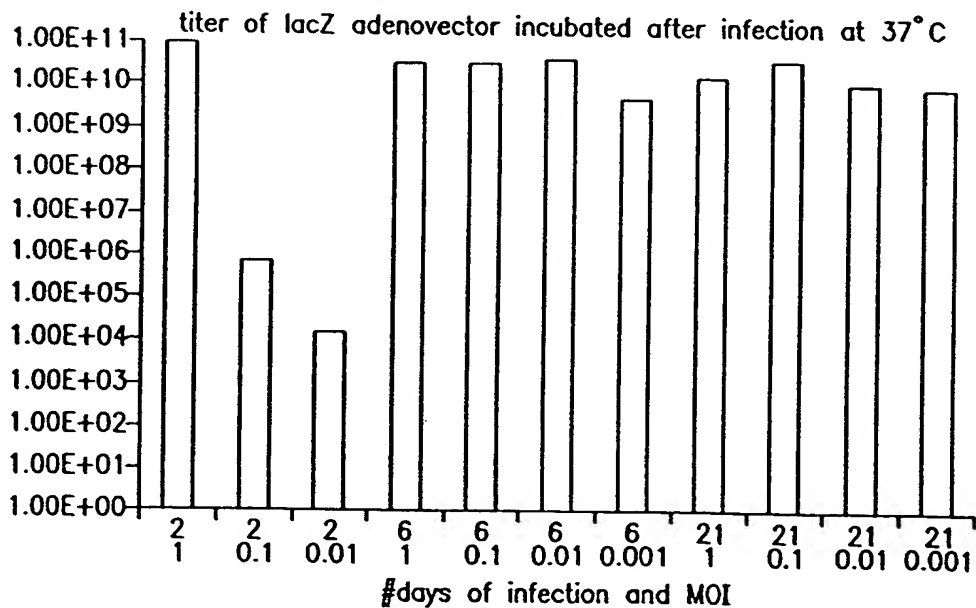
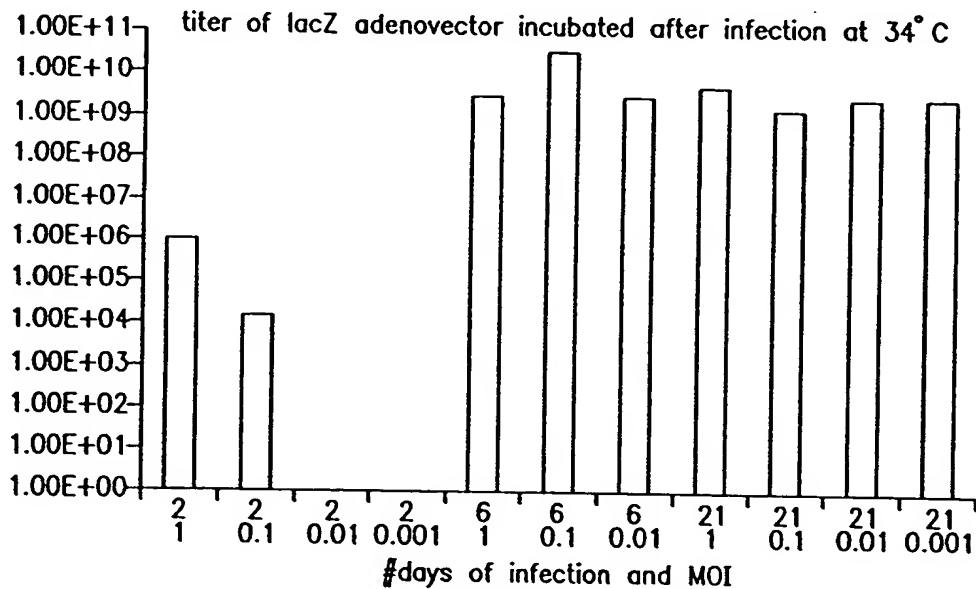
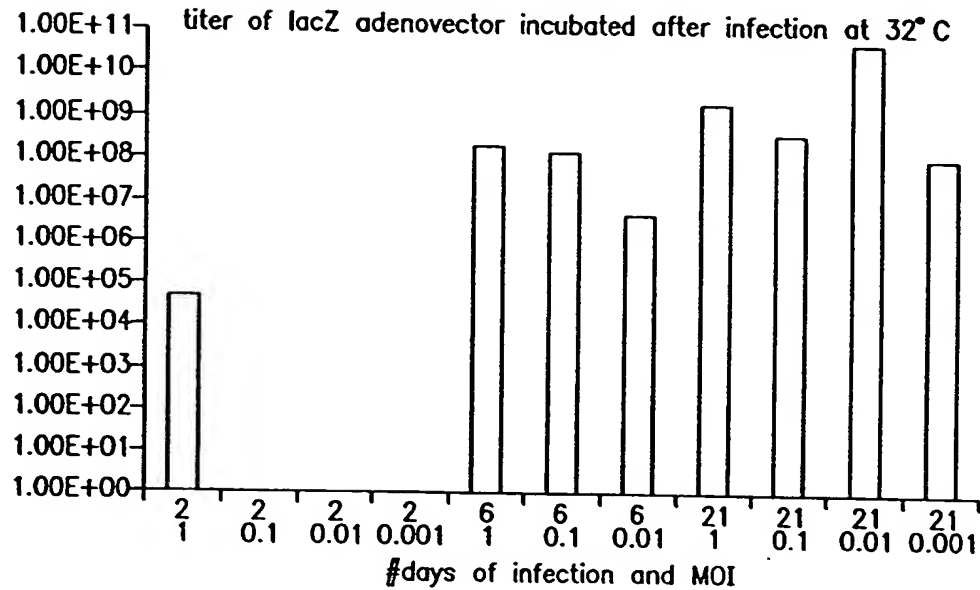
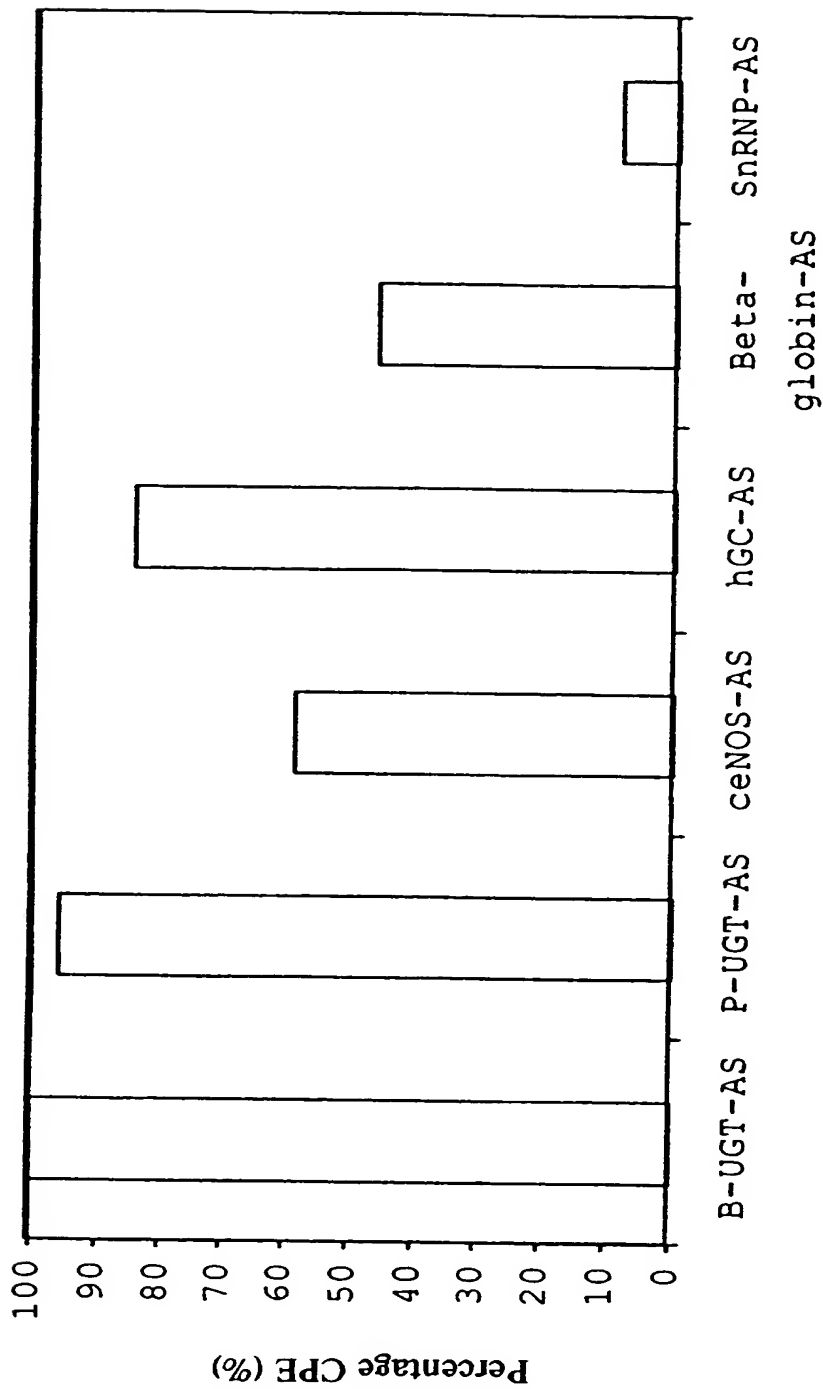


FIG. 32



AS-virus

FIG. 33

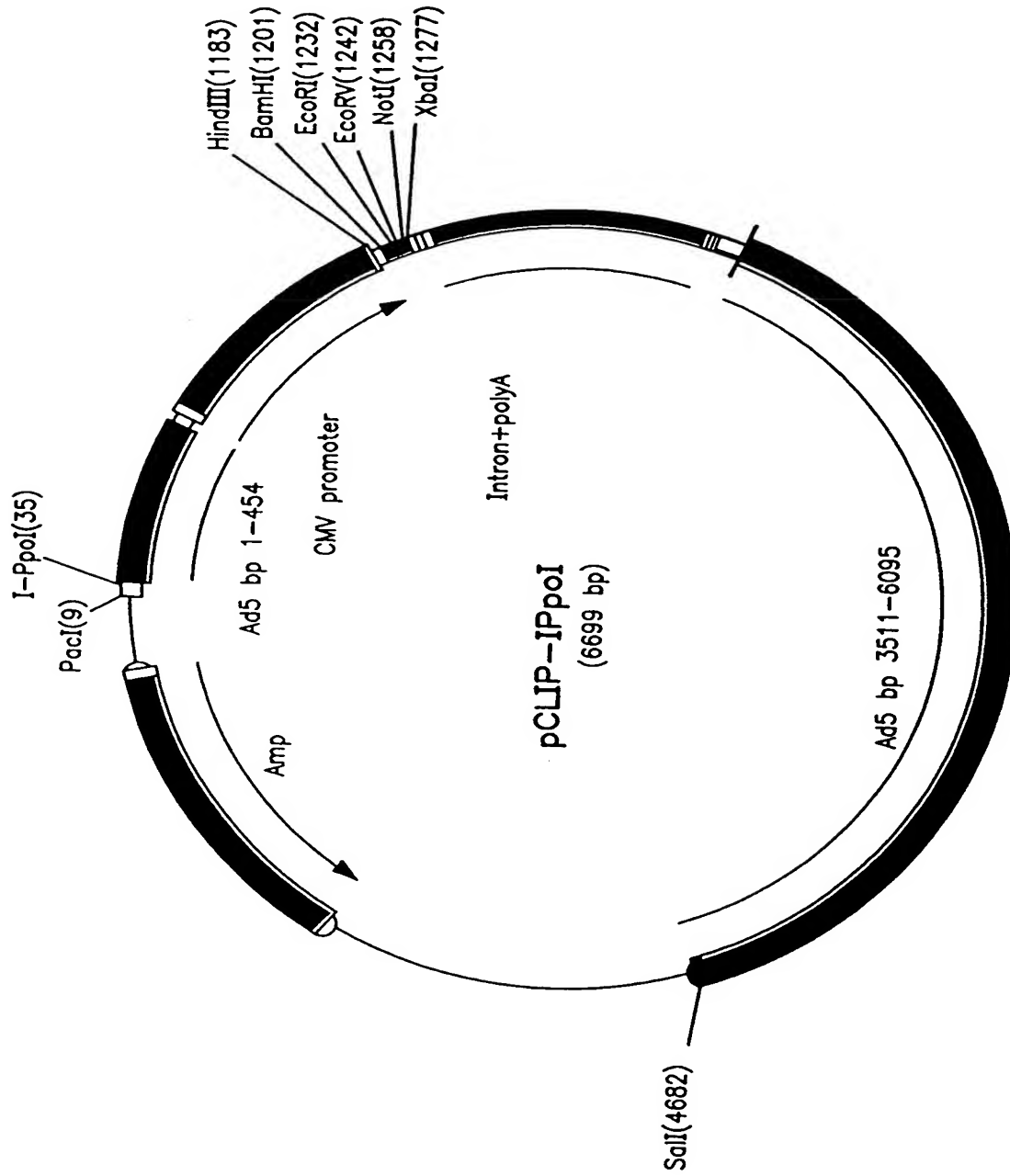


FIG. 34A

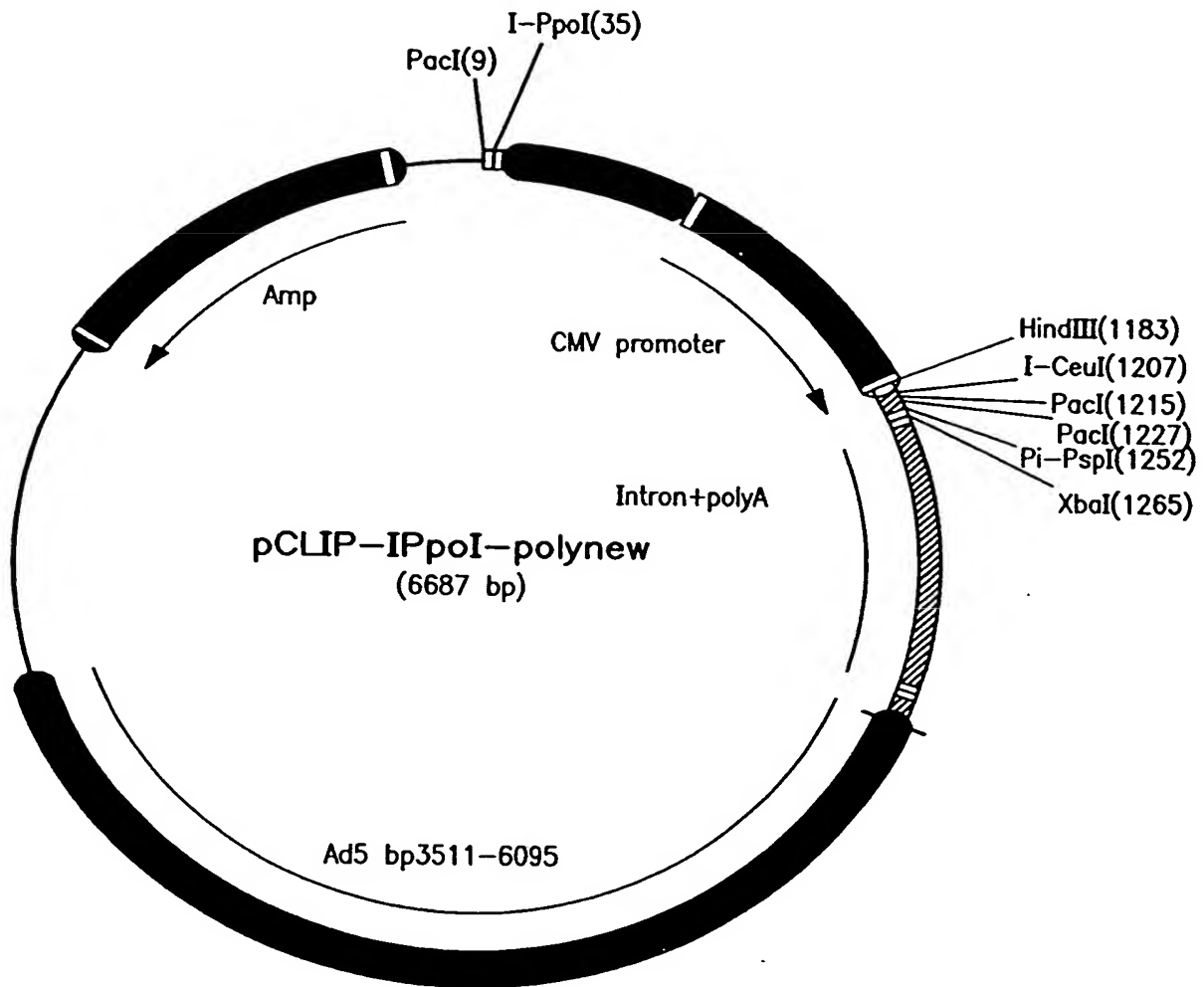


FIG. 34B

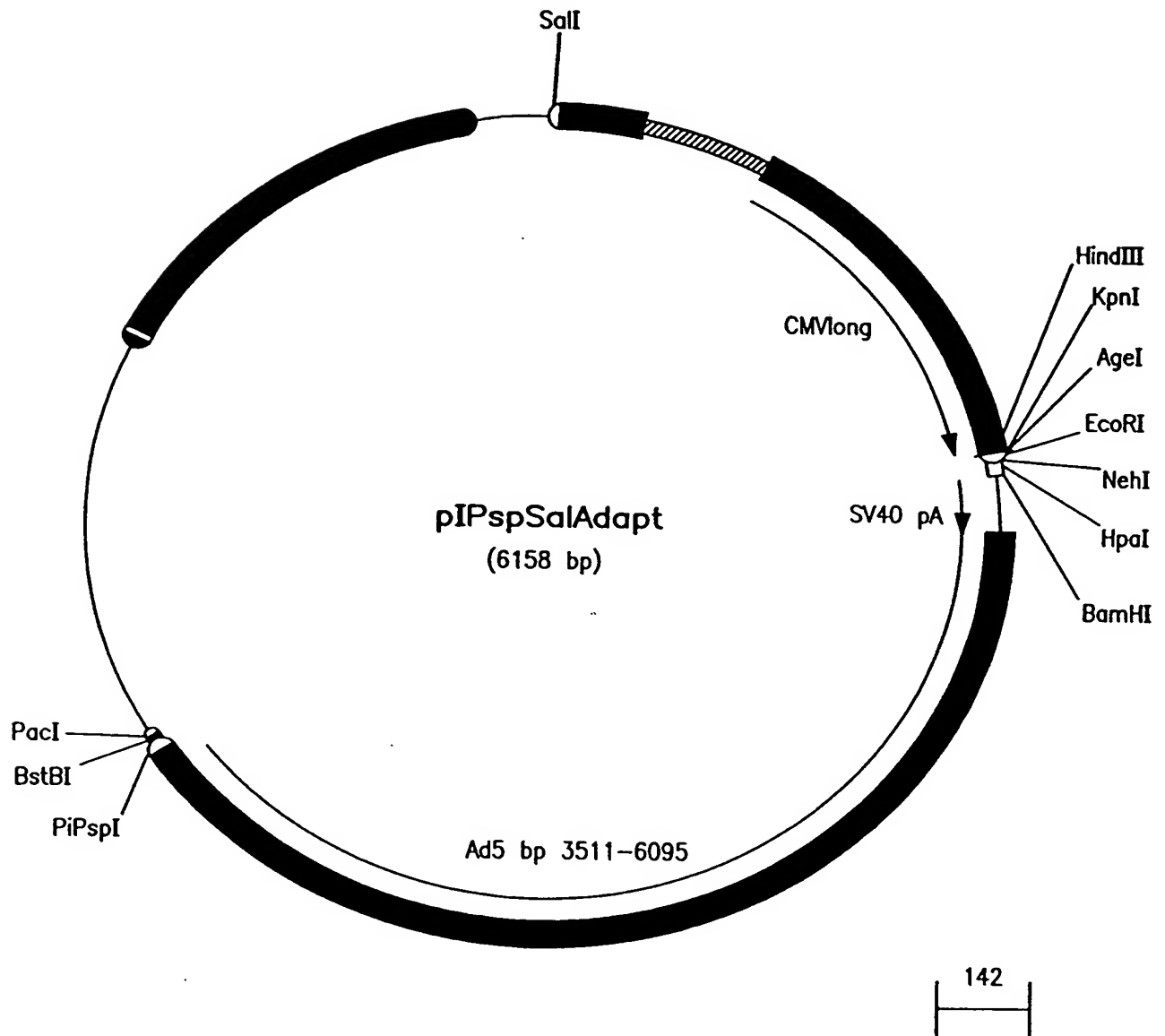


FIG. 34C

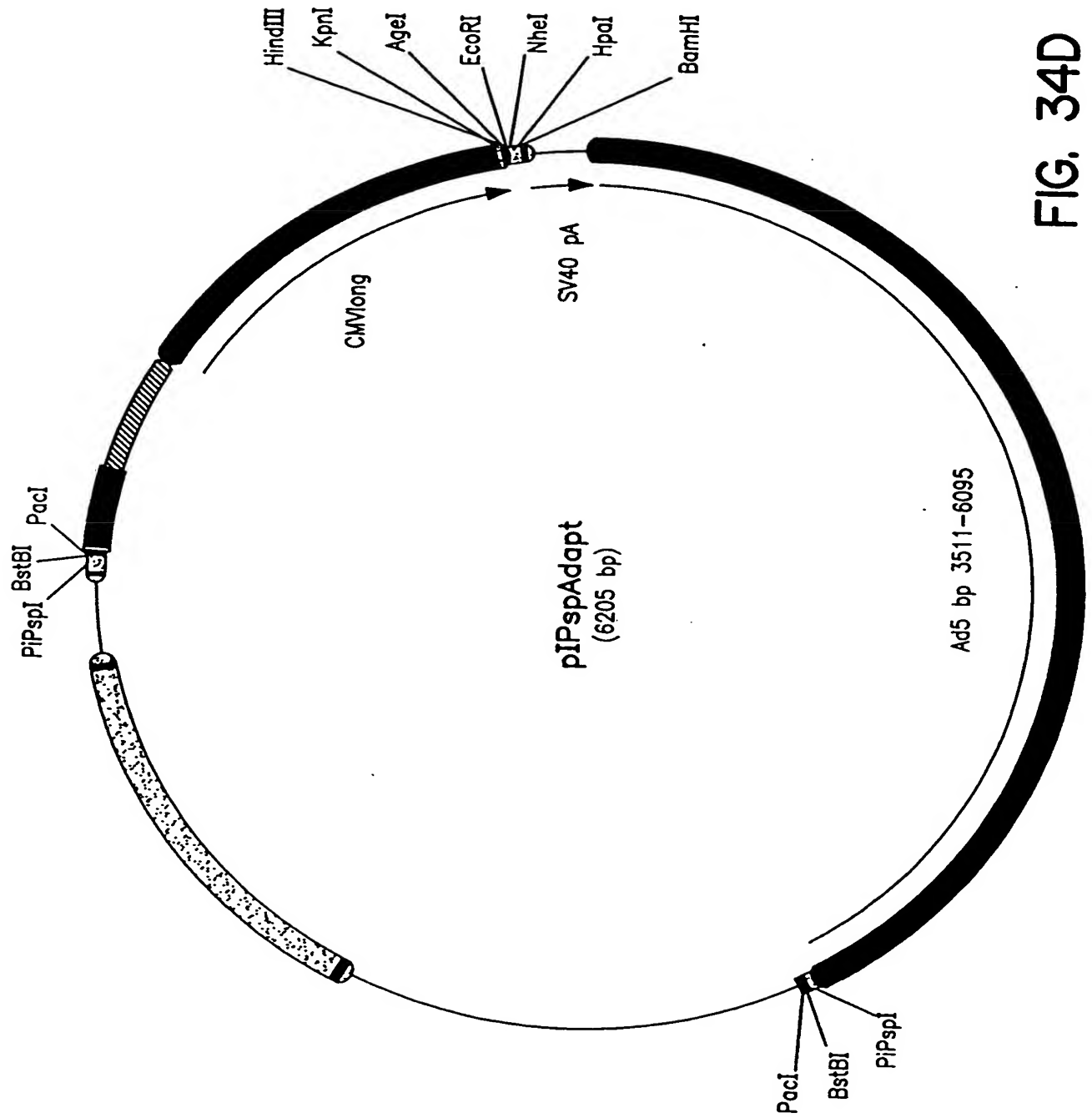


FIG. 34D

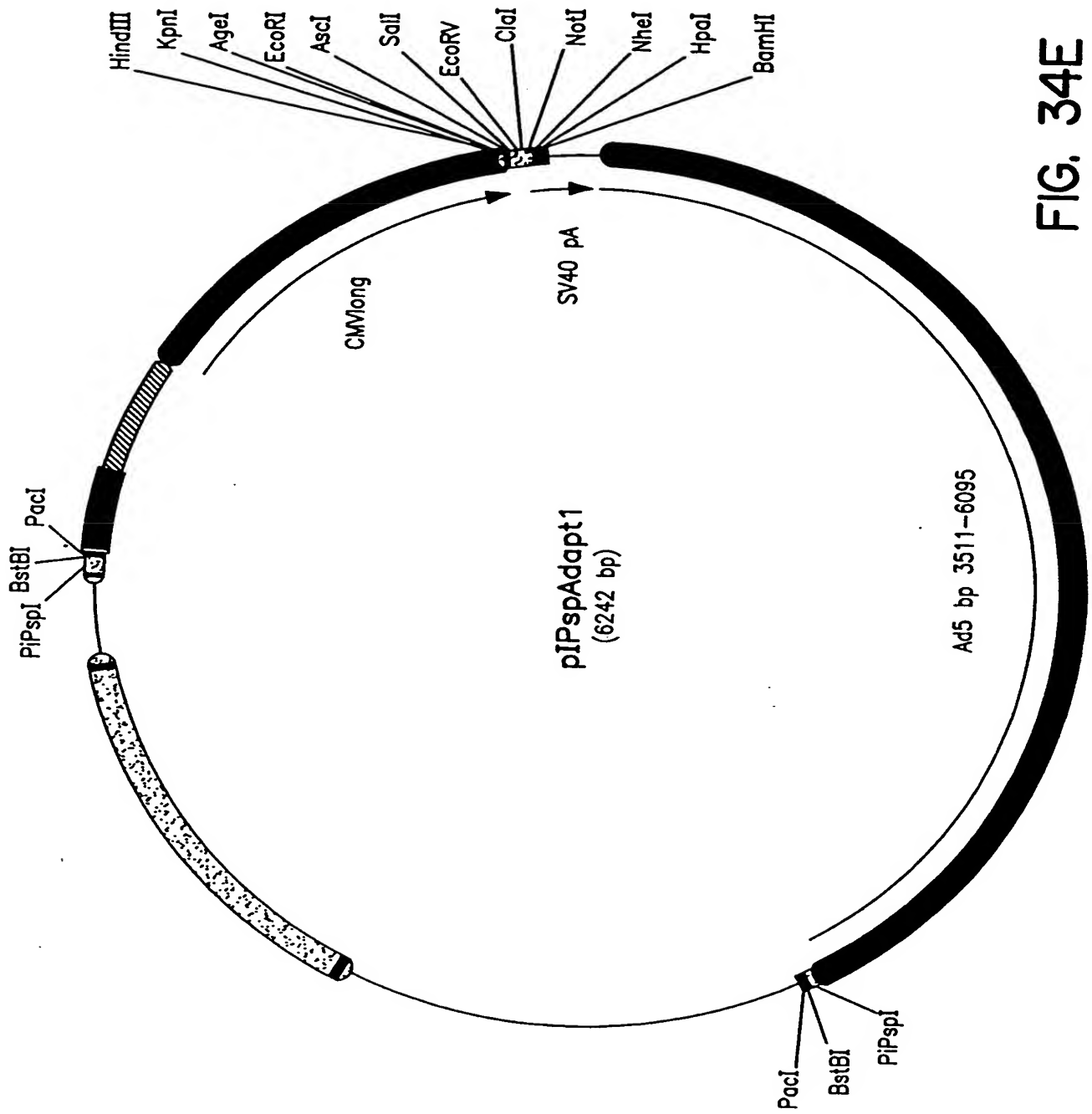


FIG. 34E

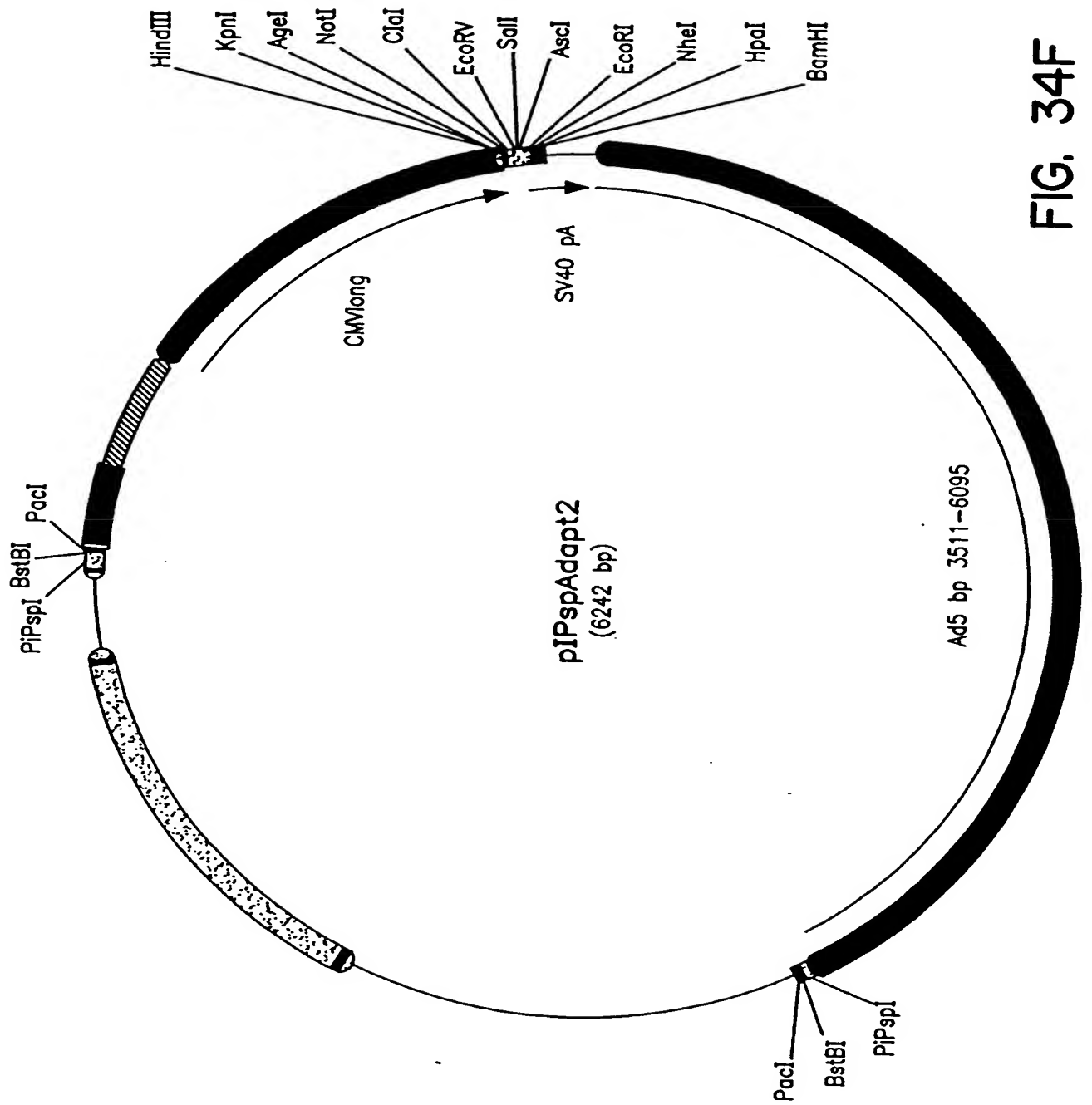


FIG. 34F

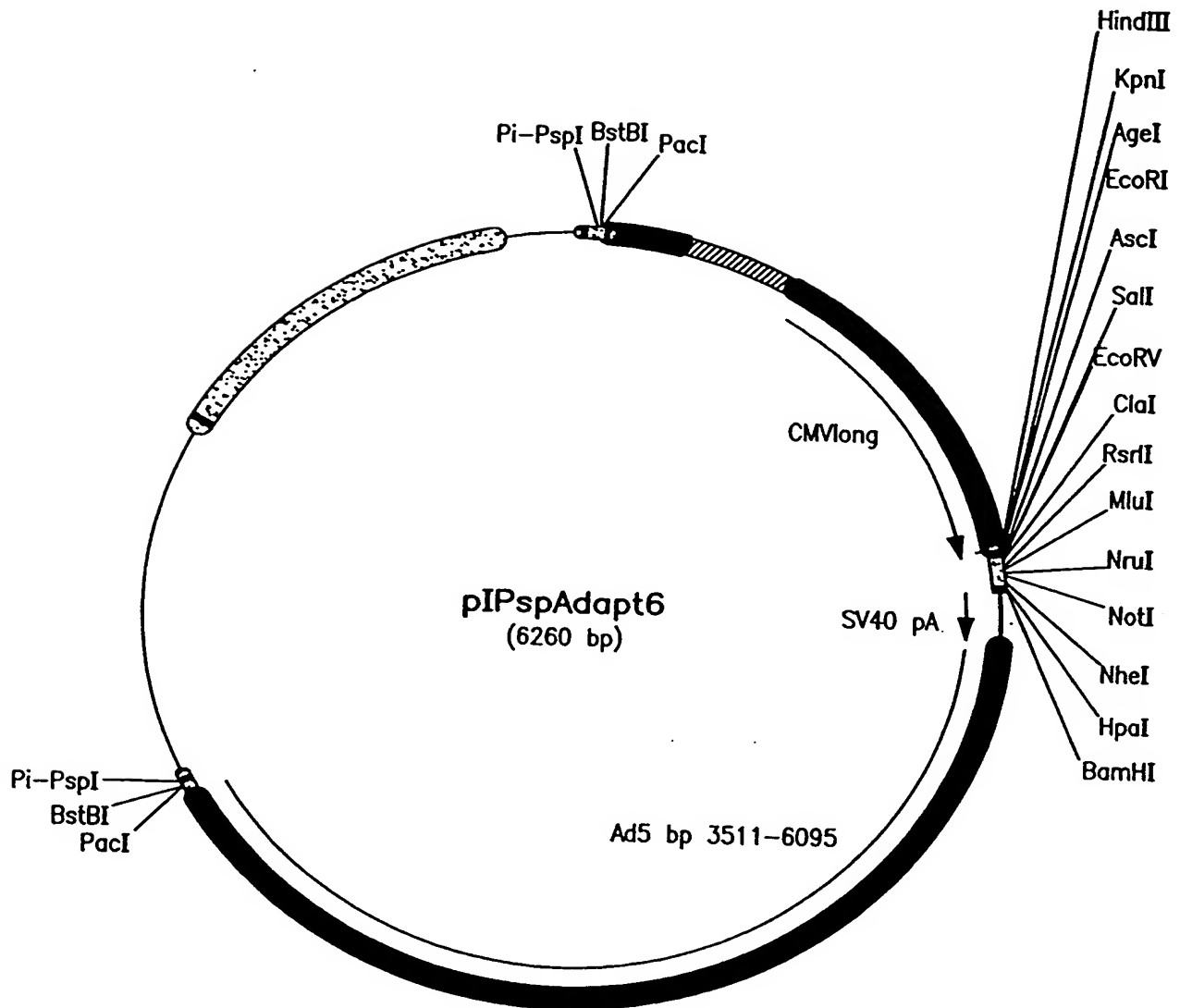


FIG. 34G

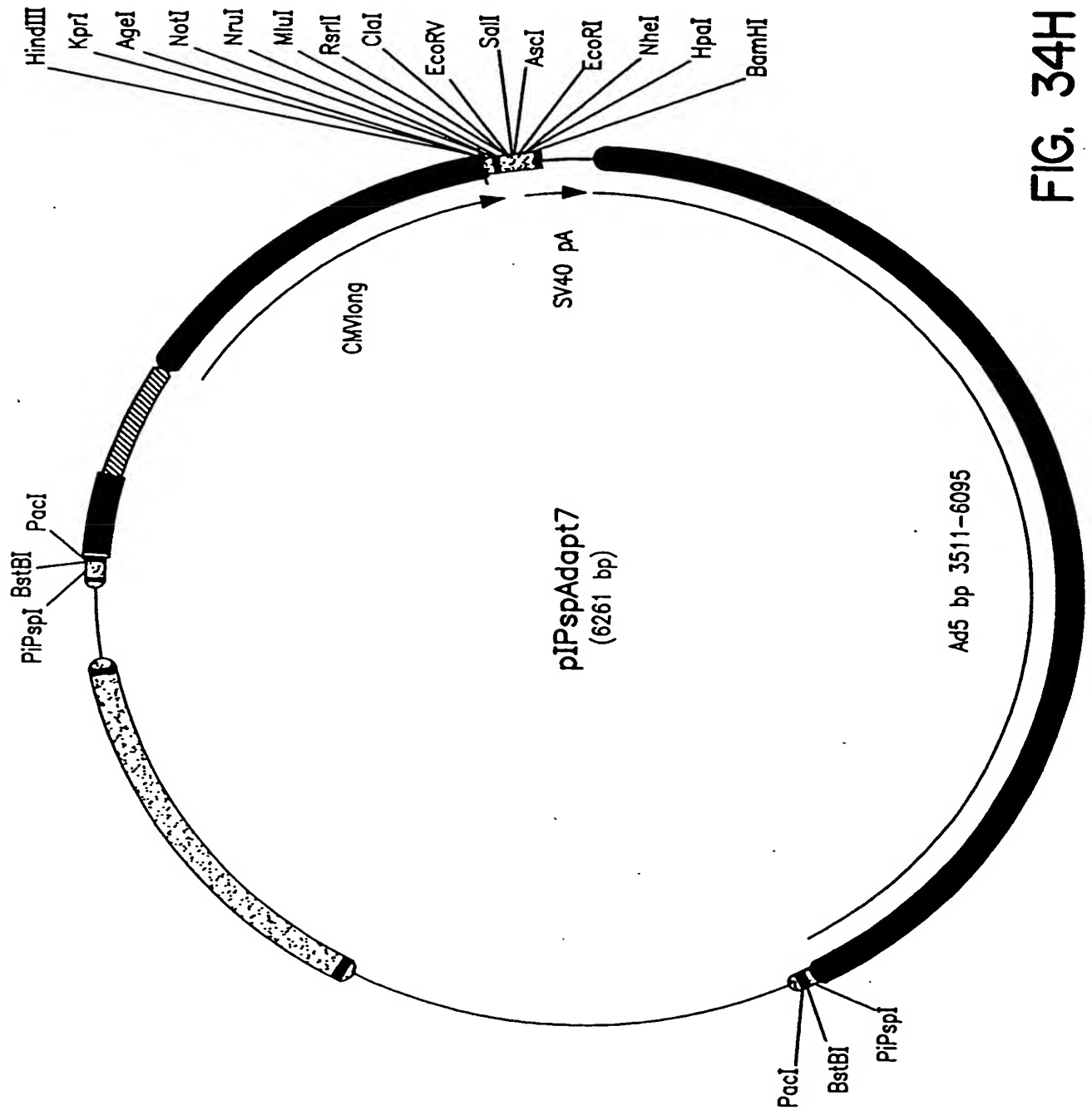


FIG. 34H

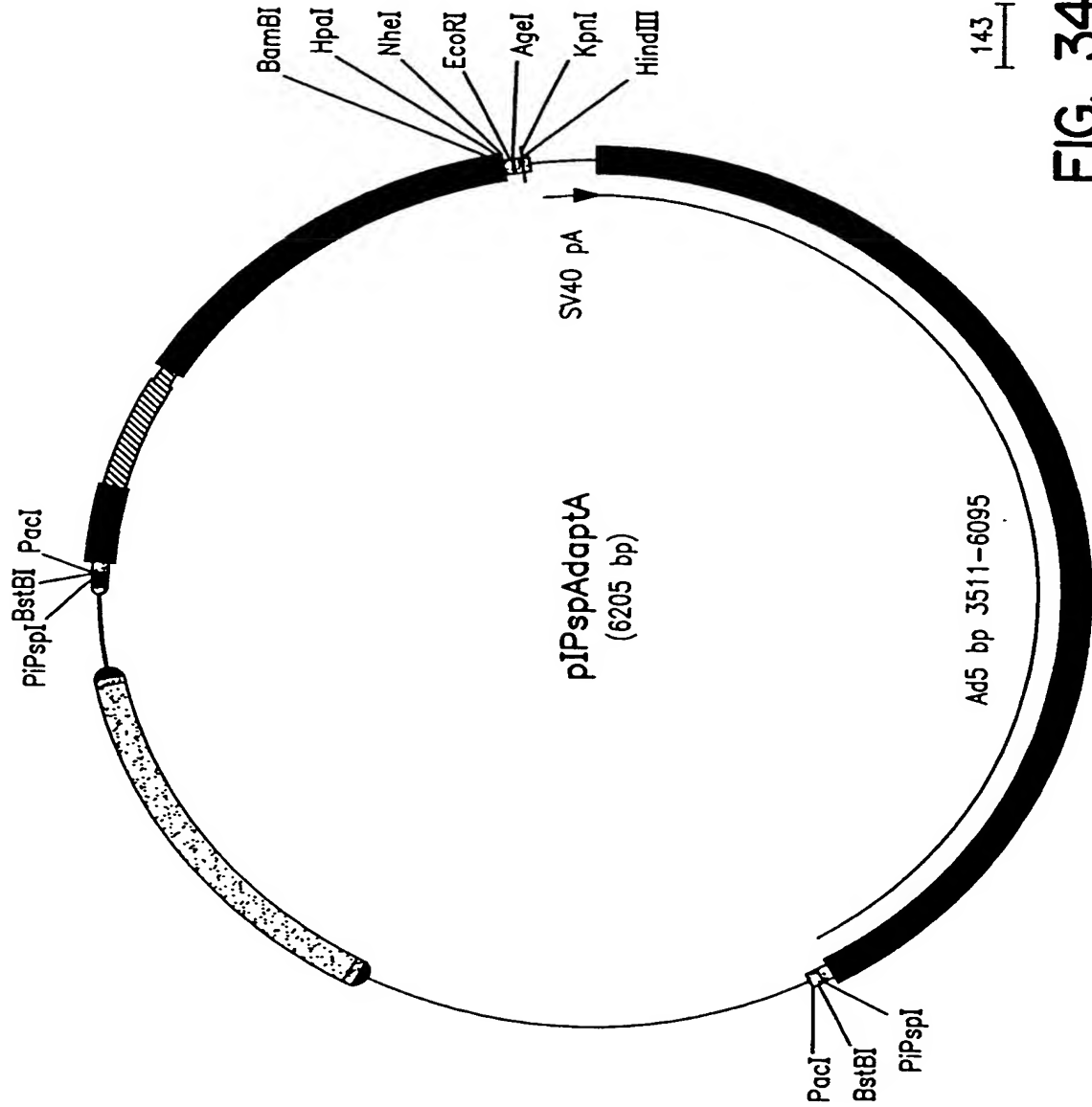


FIG. 34 I

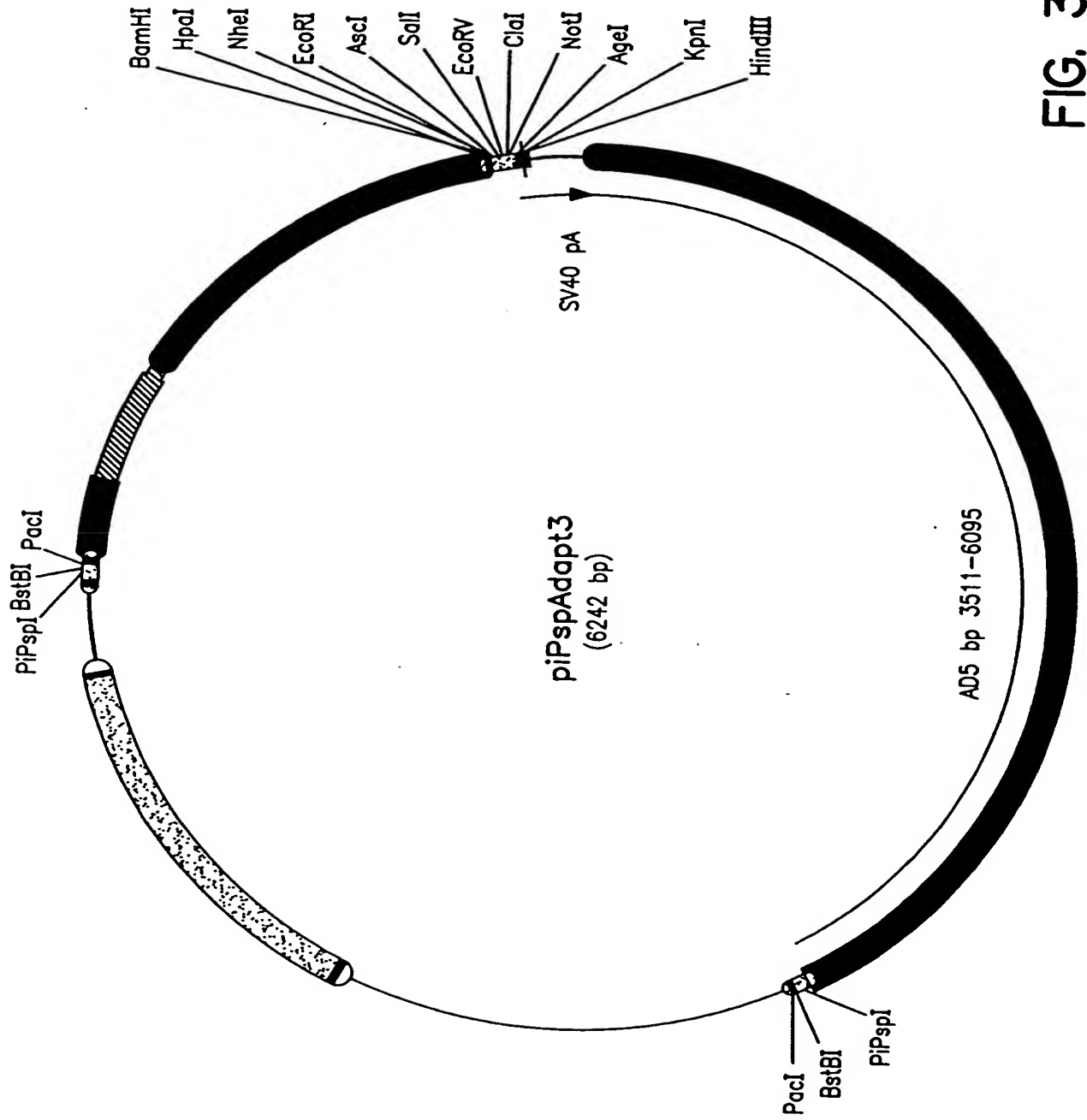


FIG. 34J

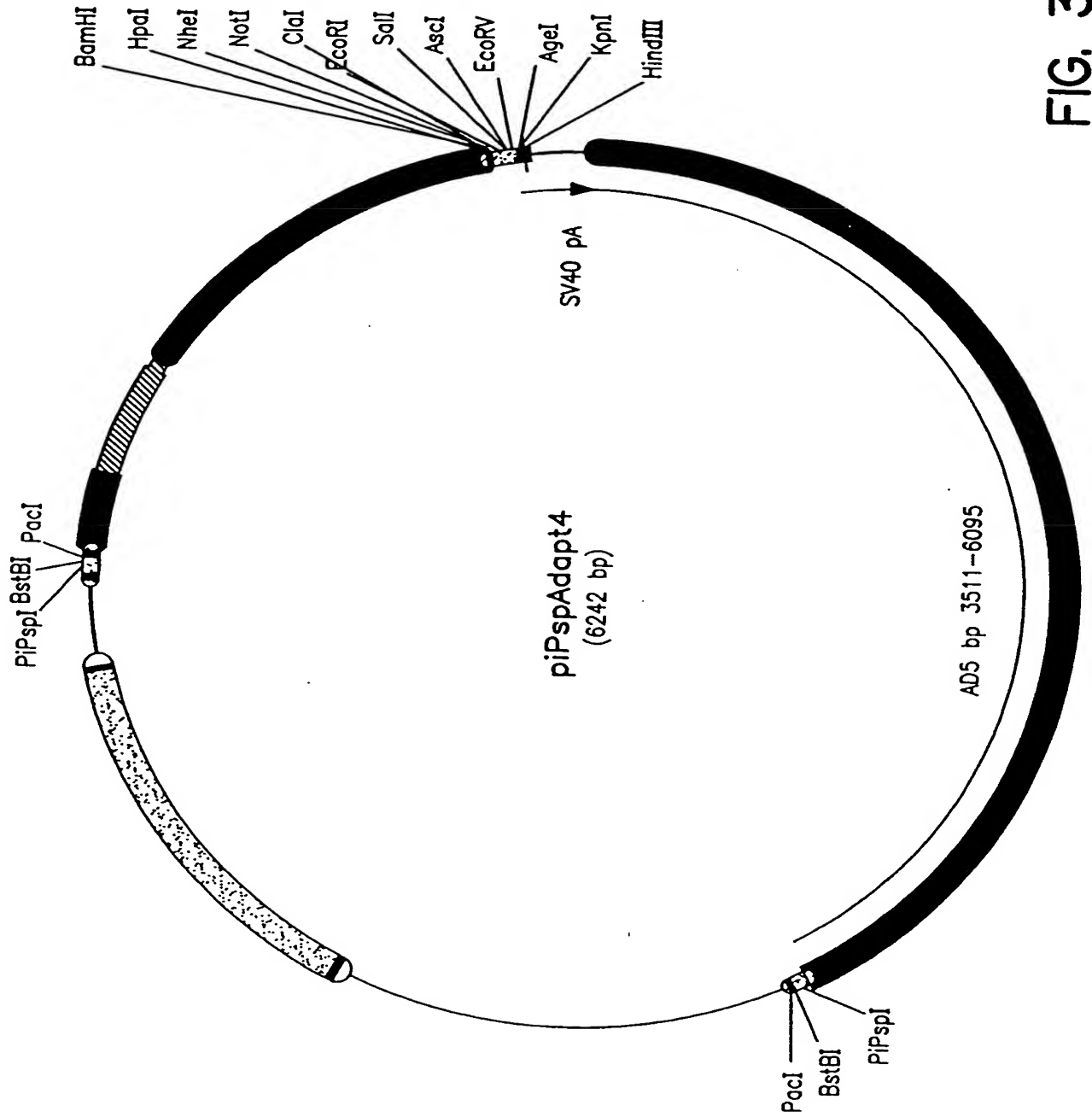


FIG. 34K

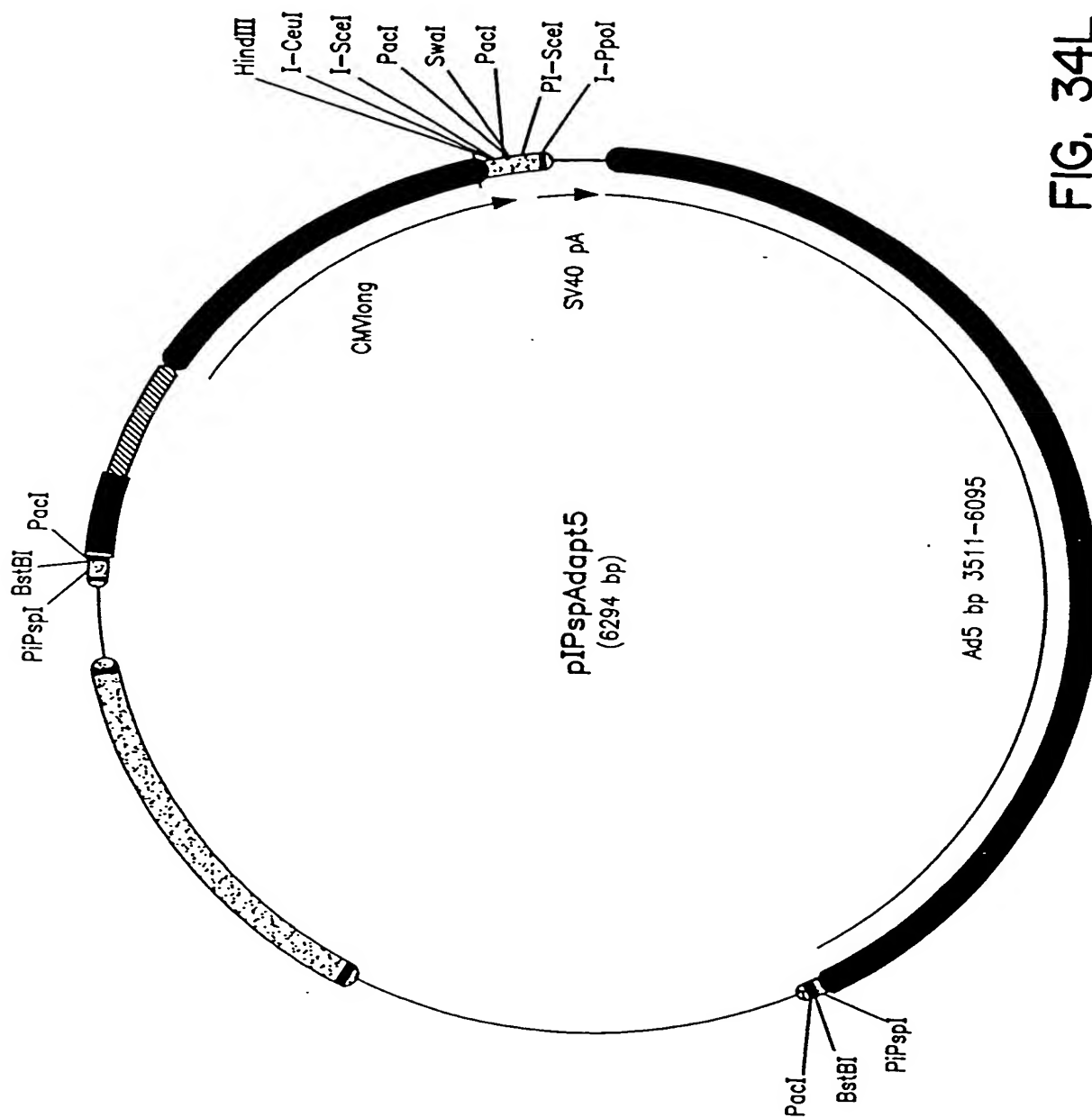
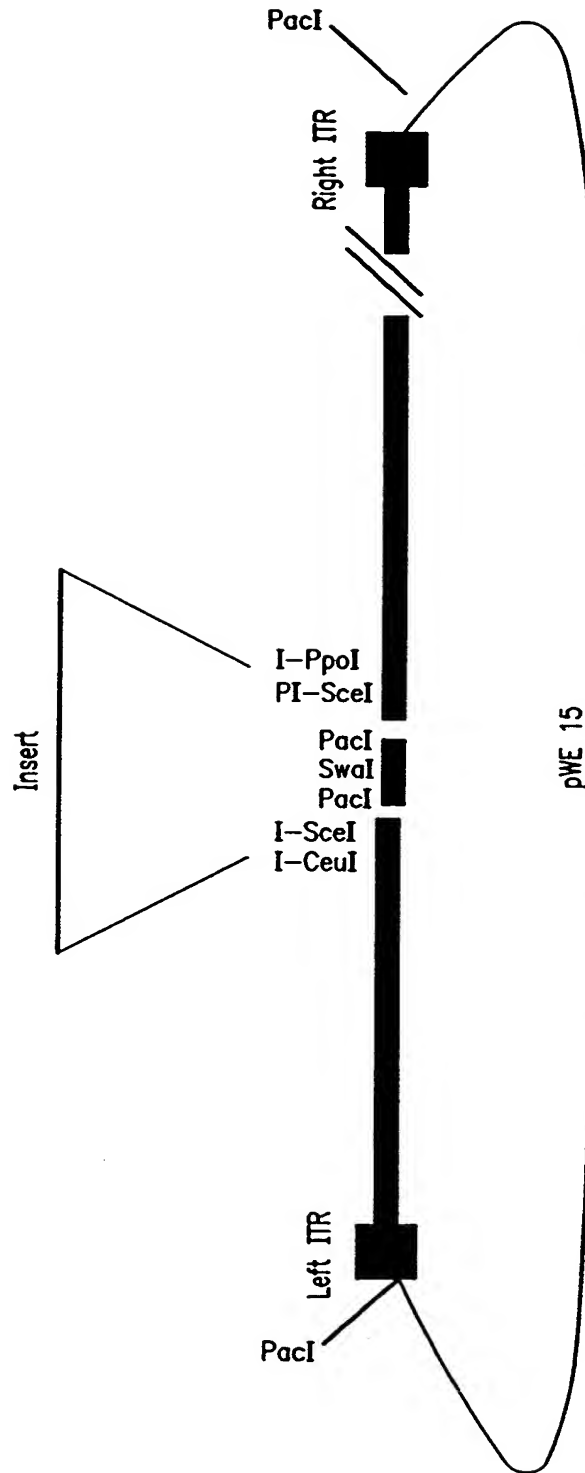


FIG. 34L

**FIG. 34M**

Relative amounts of wells with CPE after transfection of PER.C6/E2A cells with pCLIP-LacZ and the adapter plasmid pIPspAdapt2.

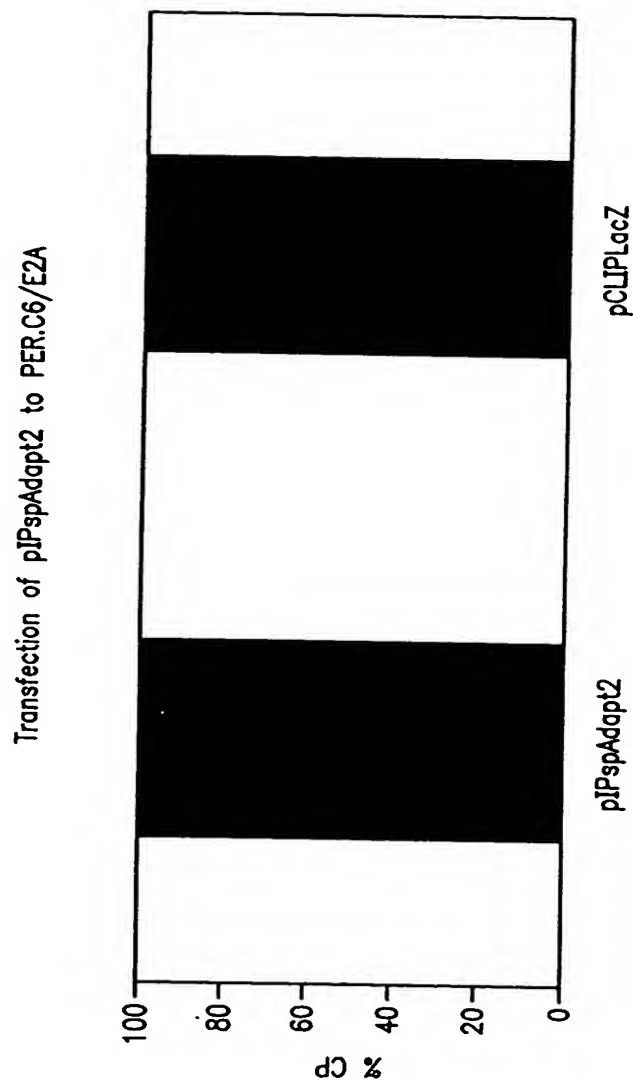


FIG. 34N

Construction total Adeno cDNA Library (1)

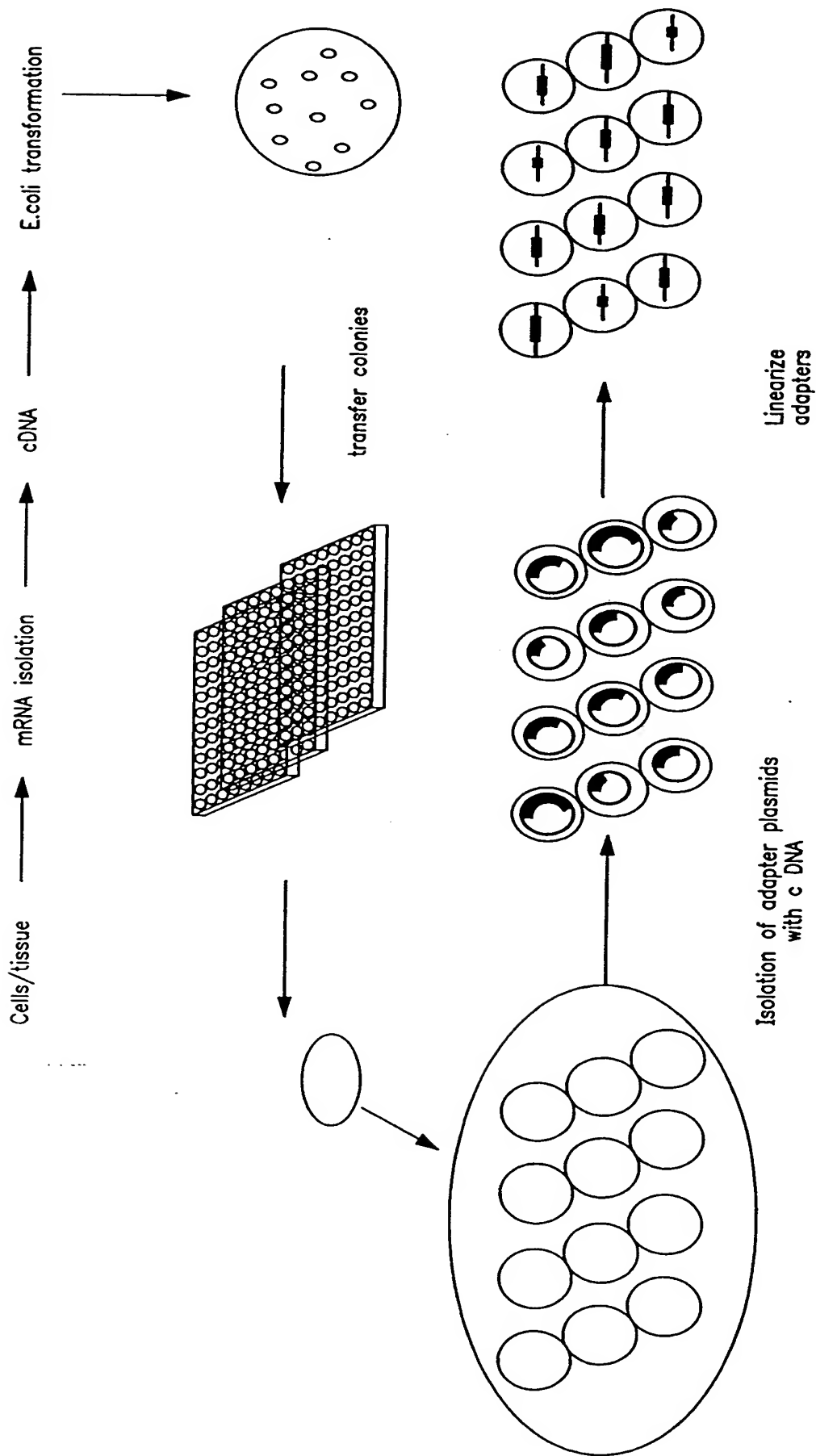


FIG. 36A

Construction total Adeno cDNA Library (II)

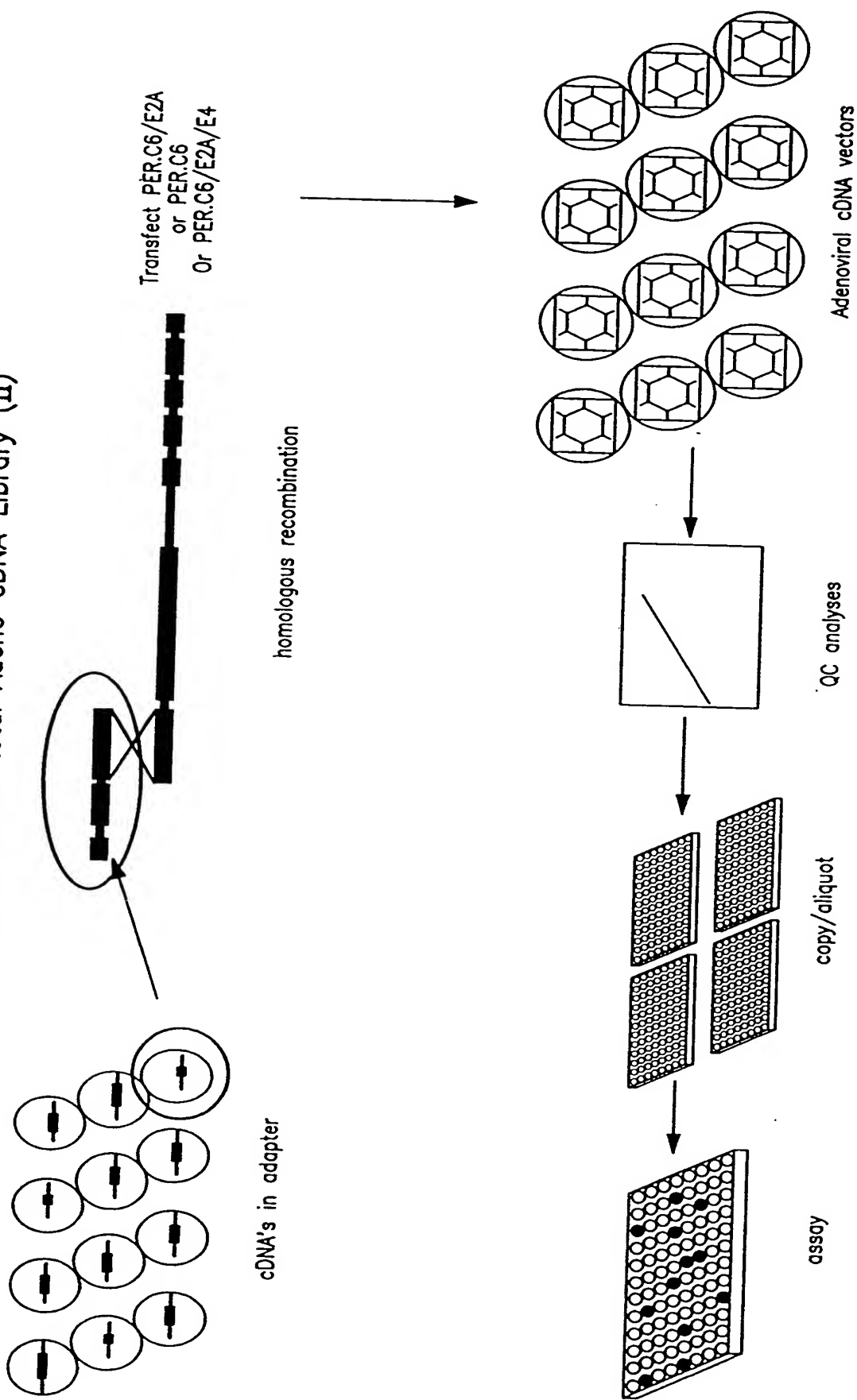


FIG. 36B

EXAMPLE 21 384 WELL PLATE IN PROGRESS

Co-transfections on 384 well plates

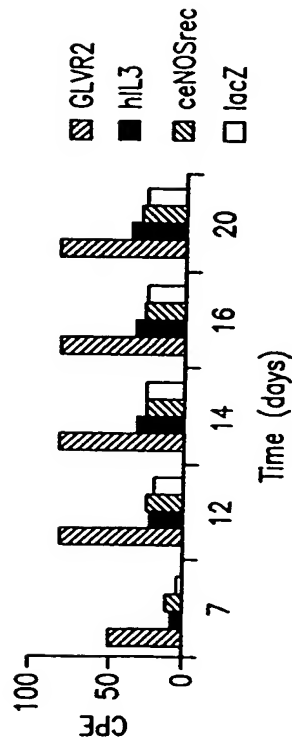


FIG. 37A

Co-transfections on 96 well plates
(control plate)

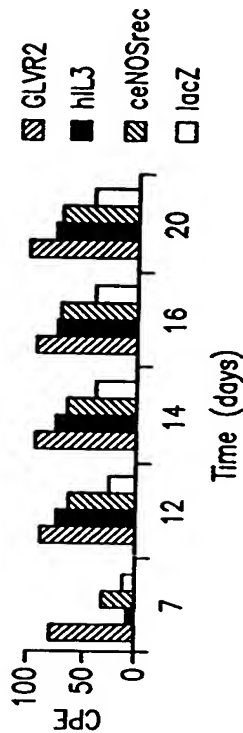


FIG. 37B

Co-transfections on 384 well plates

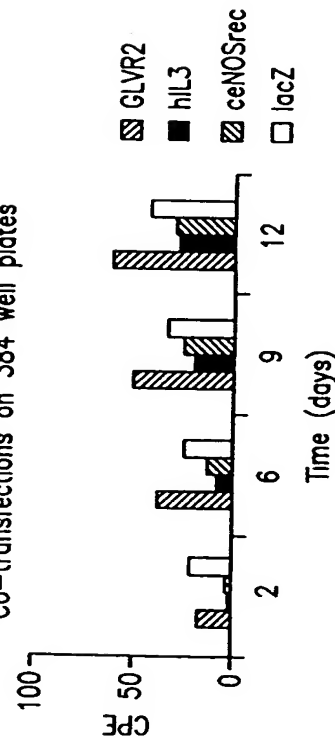


FIG. 37C

Co-transfections on 96 well plates
(control plate)

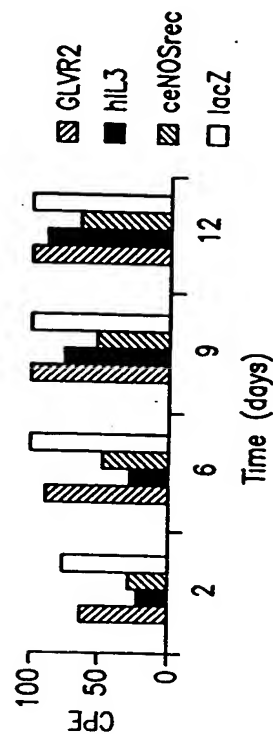


FIG. 37D

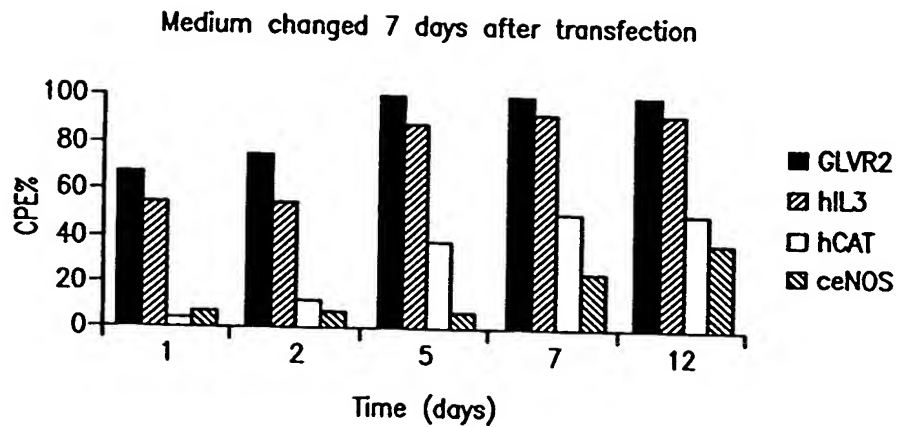


FIG. 38A

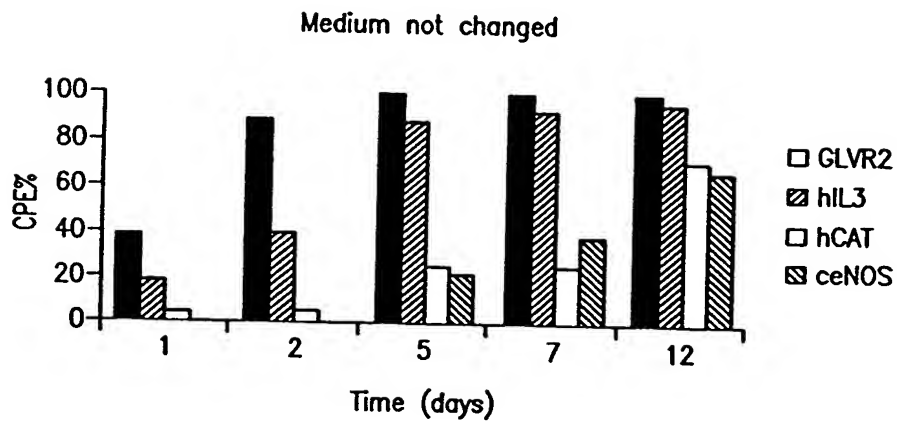


FIG. 38B

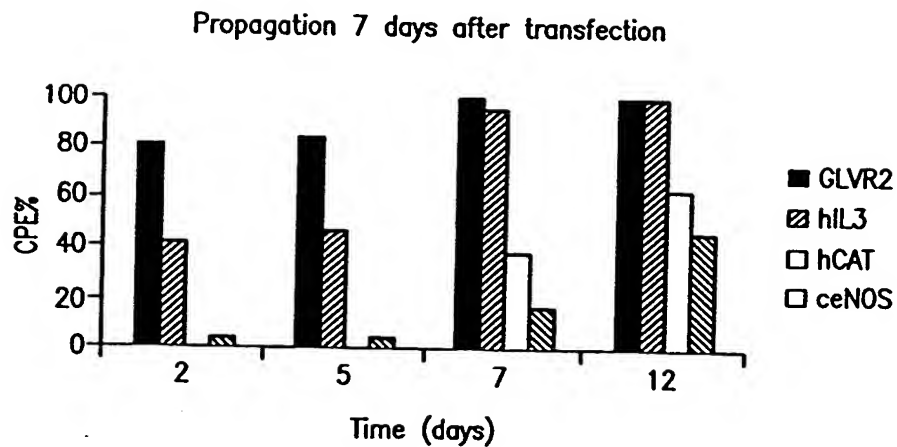


FIG. 38C

Cell titration experiment #1

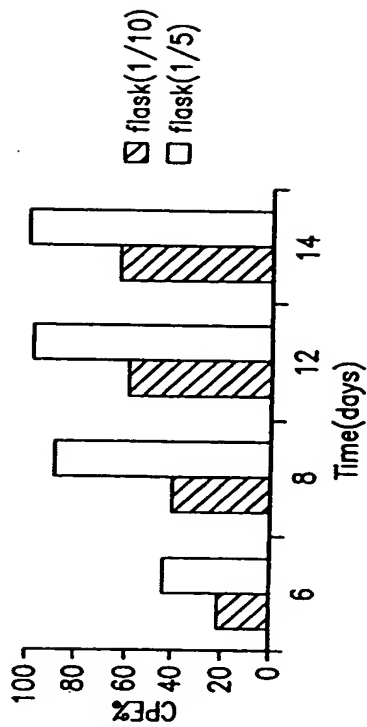


FIG. 39A

Cell titration experiment #2

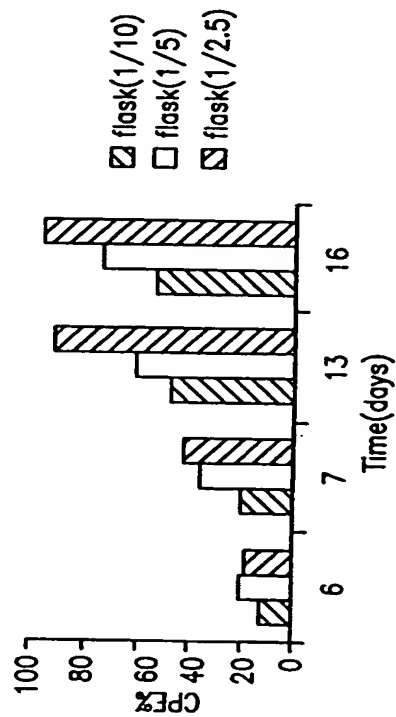


FIG. 39B

Cell titration experiment #3

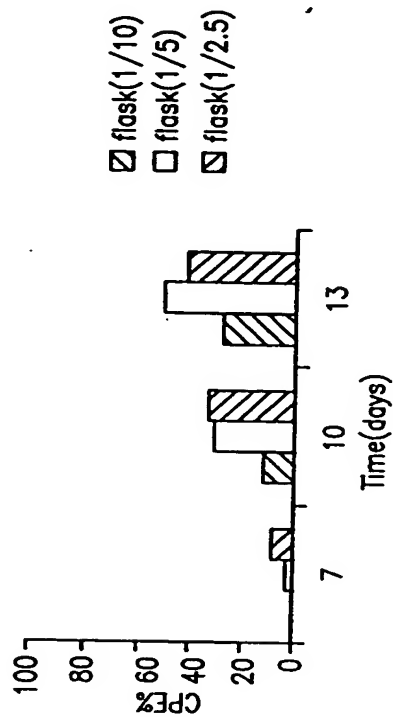


FIG. 39C

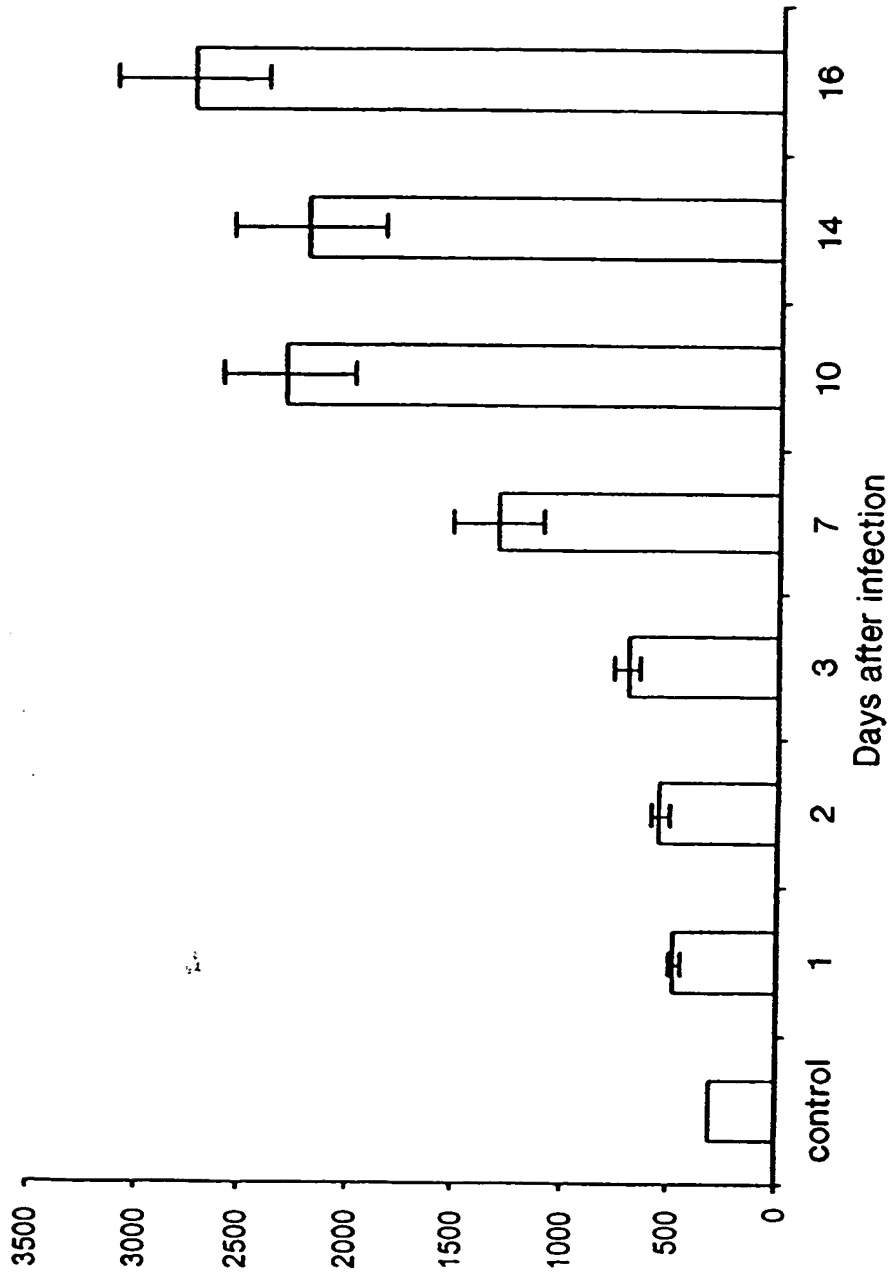


FIG. 40

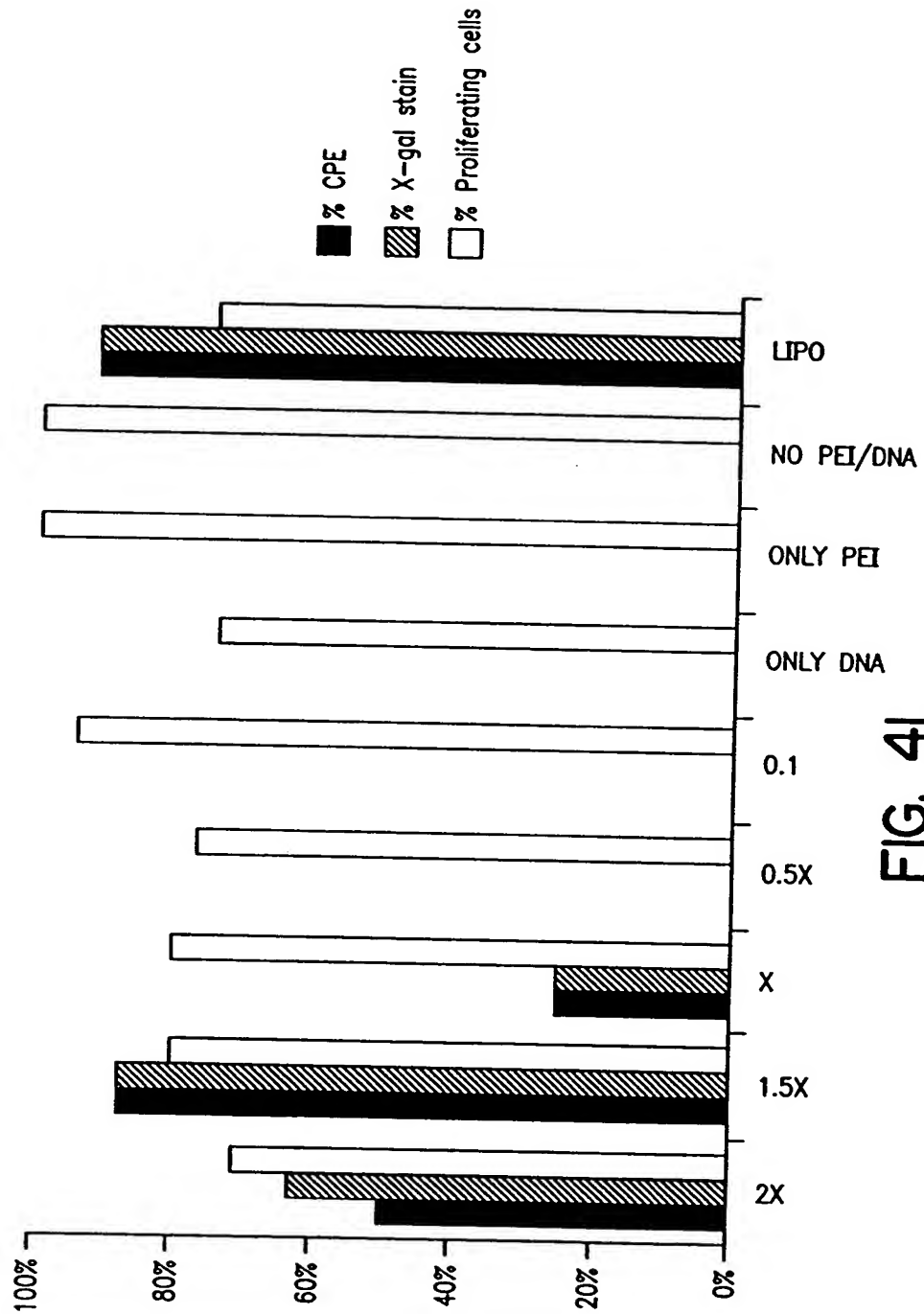


FIG. 4I

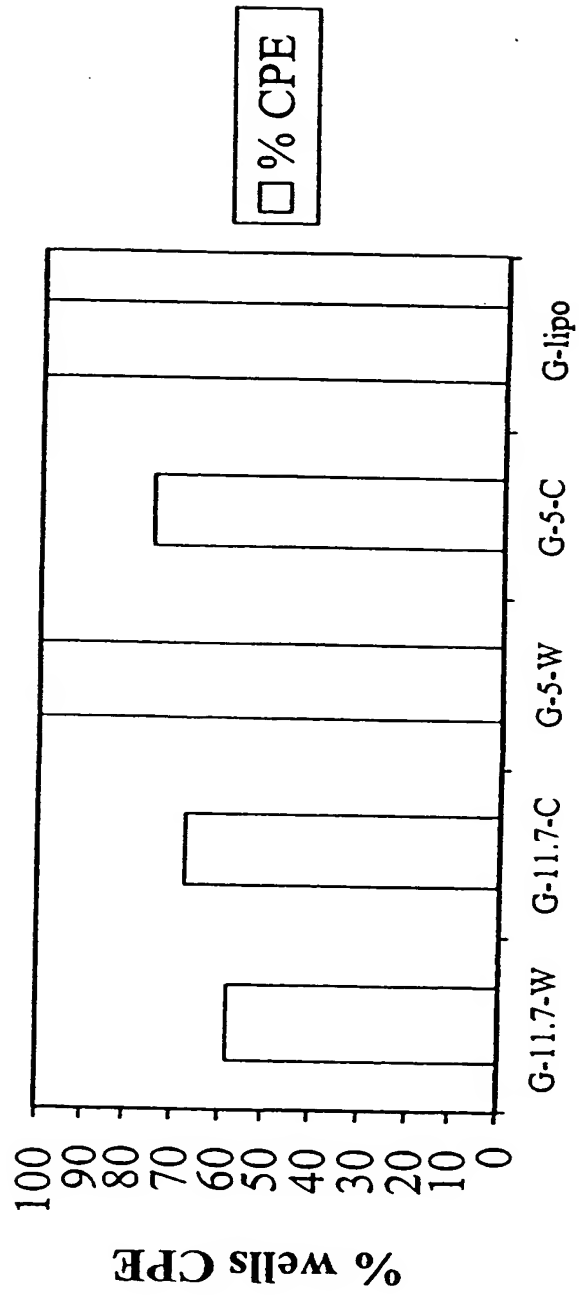


FIG. 42

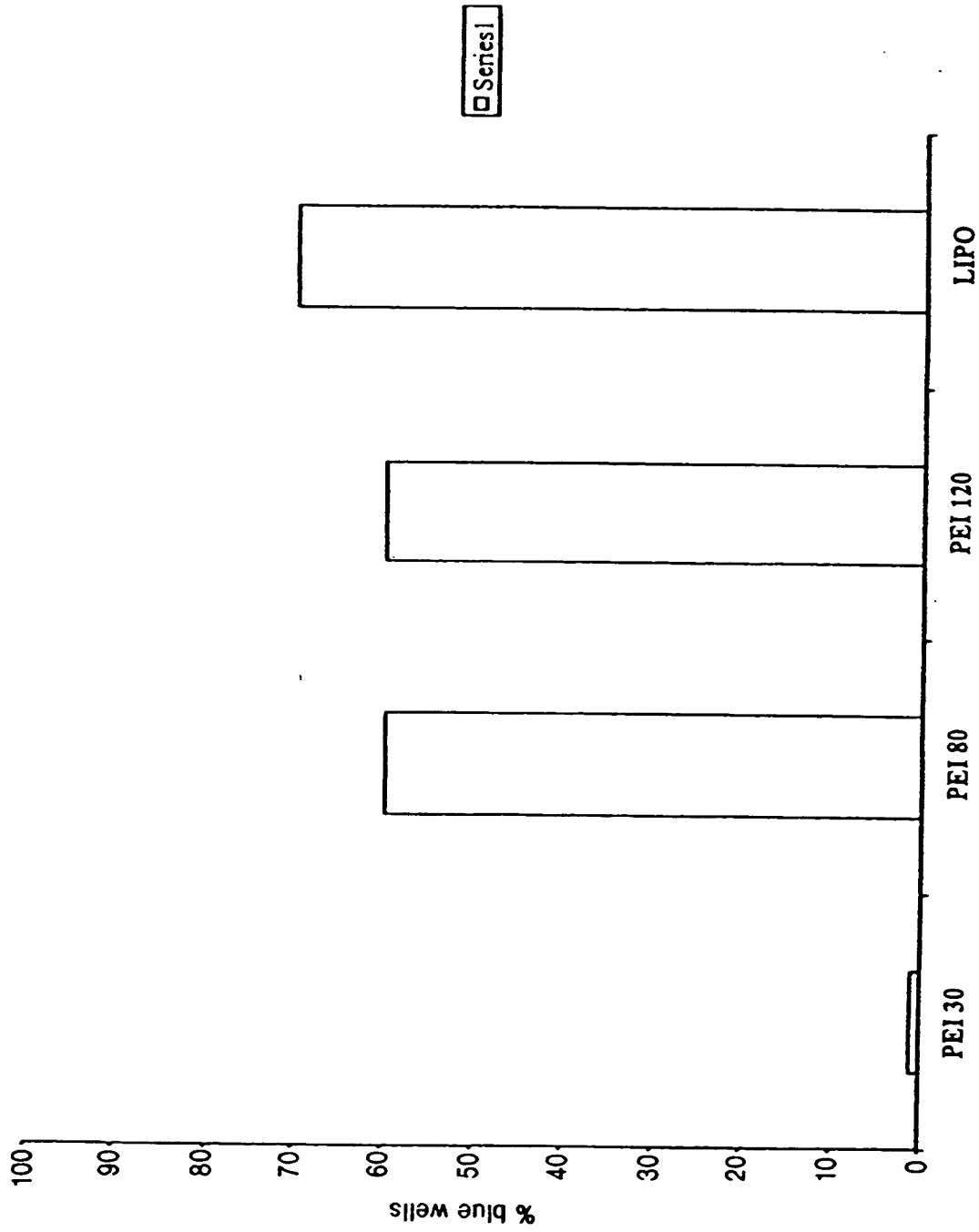


FIG. 43

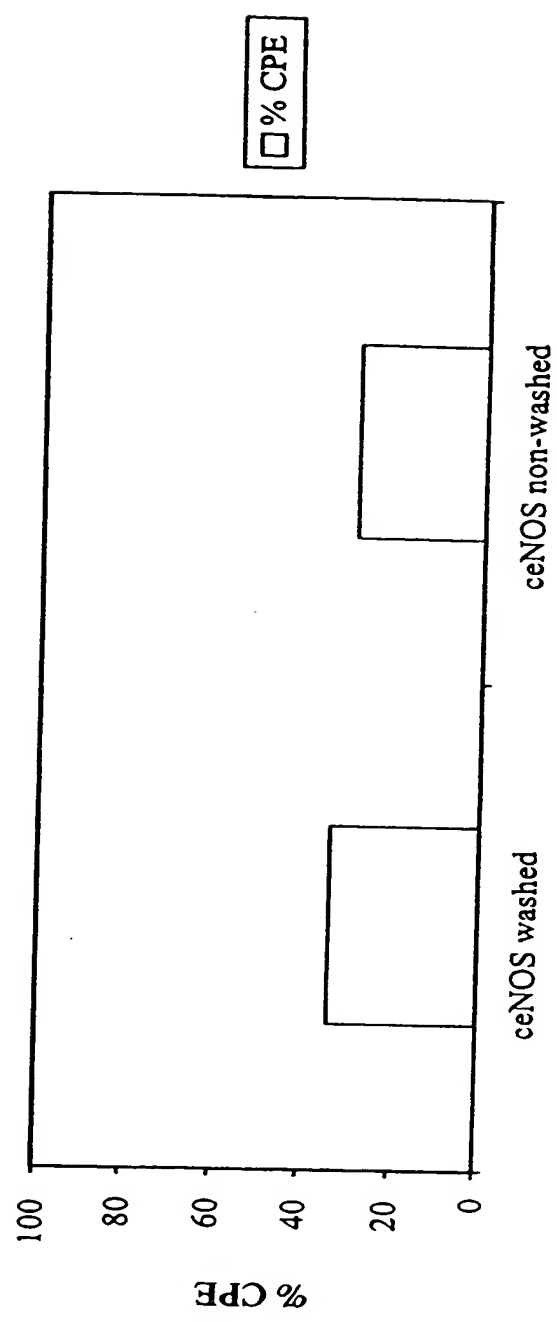


FIG. 44

Figure 45

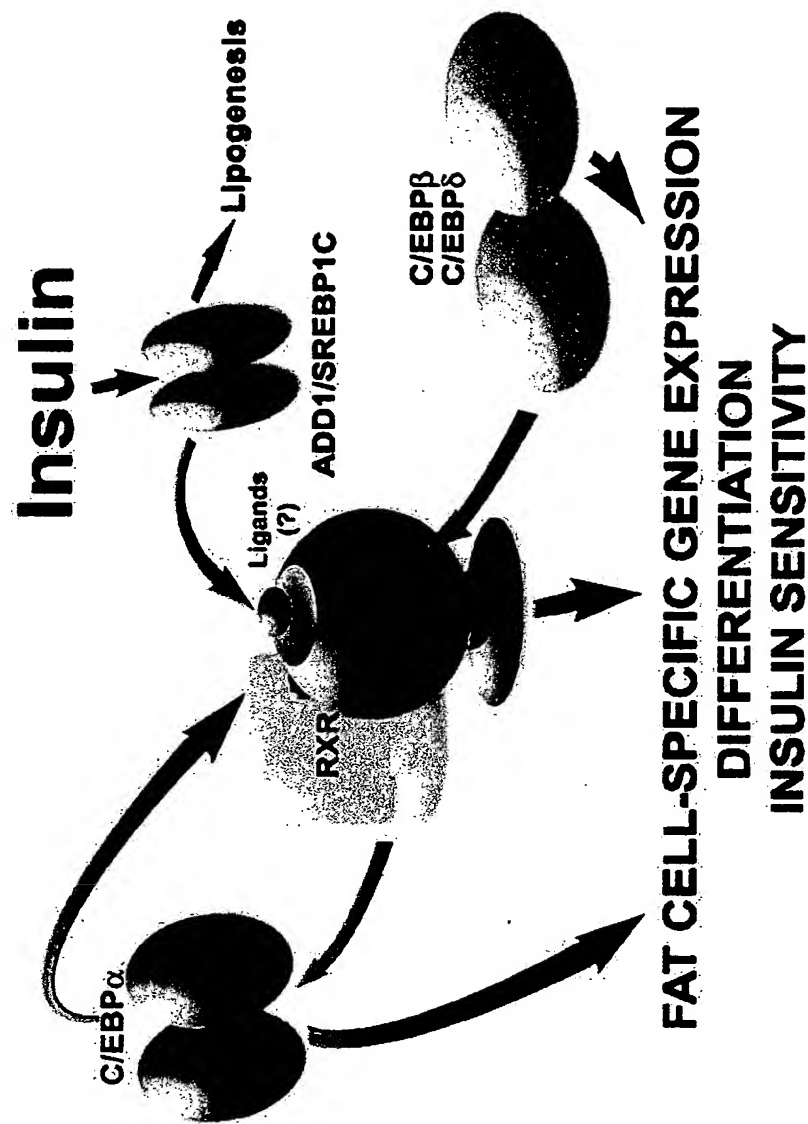


Figure 46

Transduction of hCAR⁻ cells with Ad5

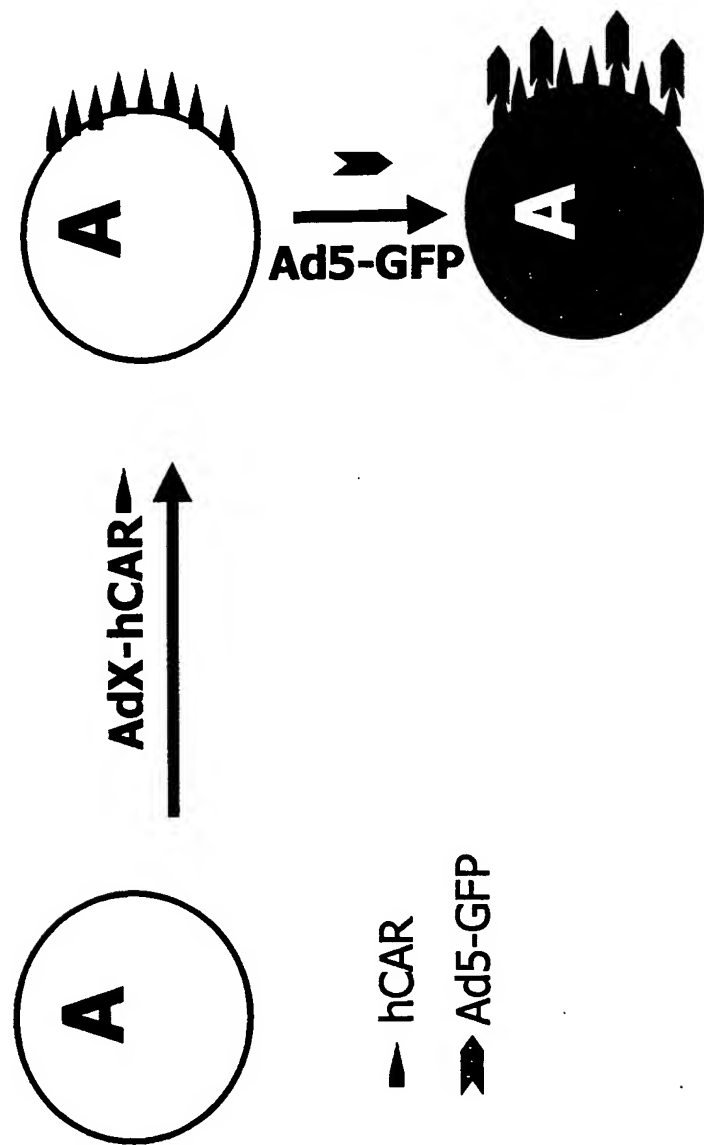
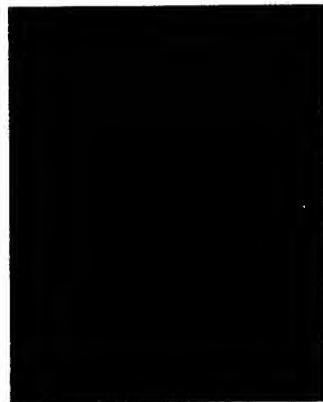


Figure 47

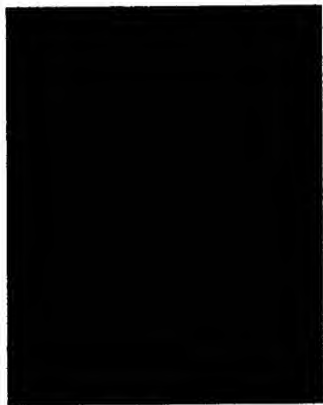
Infection of human primary pre-adipocytes using Ad5C01 and Ad5C20 fiber-modified viruses

Ad5C01-eGFP



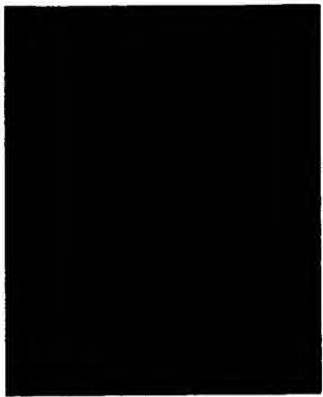
MOI:

50,000



MOI:

10,000



MOI:

2,000

Ad5C20-eGFP

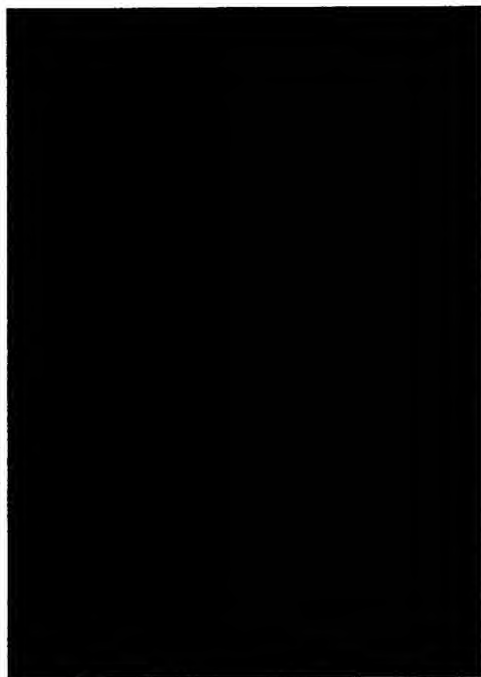


Ad5C01-Empty

+

Ad5C20-hCAR

A

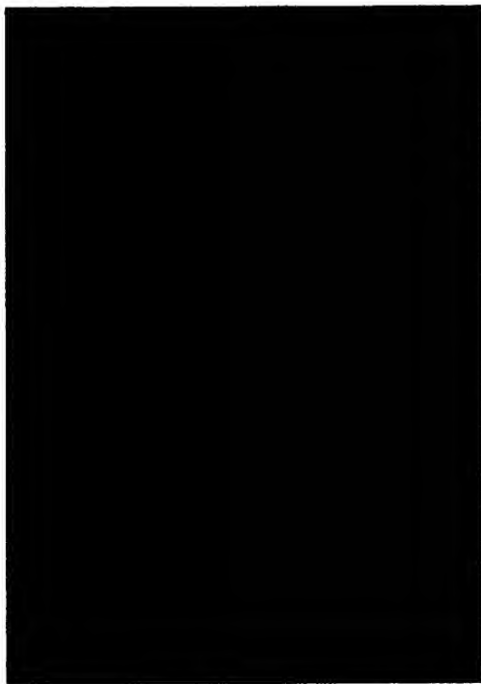


Ad5C01-PPAR γ

+

Ad5C20-hCAR

C



B



D

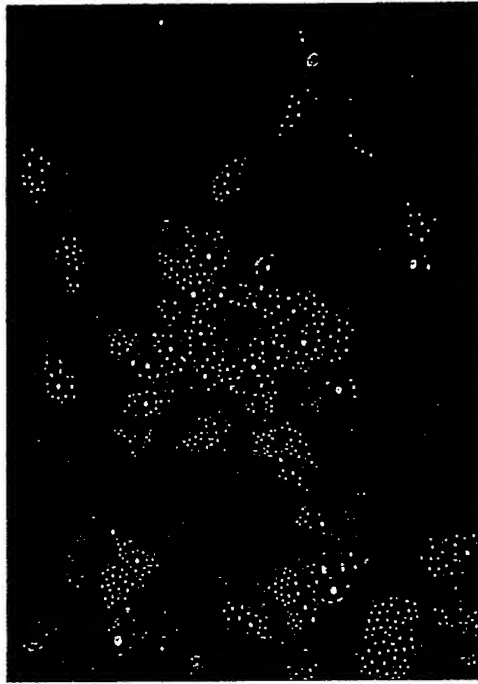
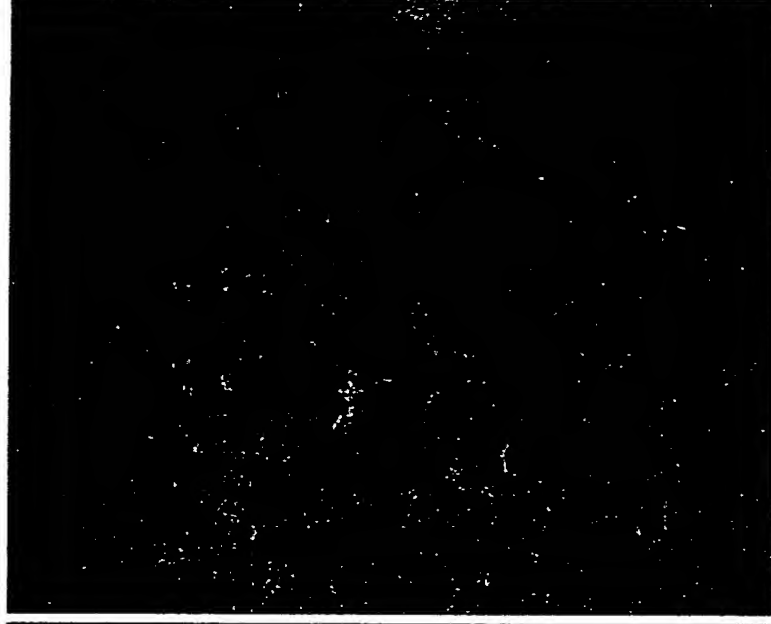
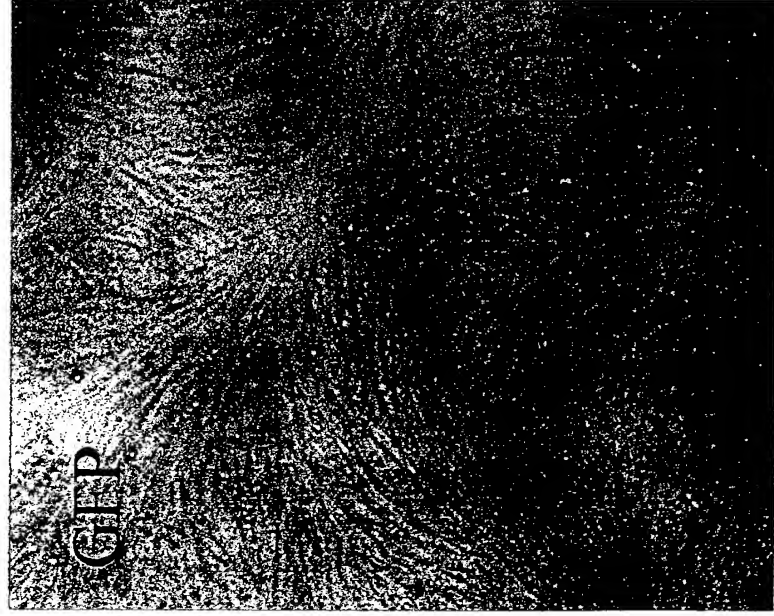


Figure 48

Adipocyte differentiation

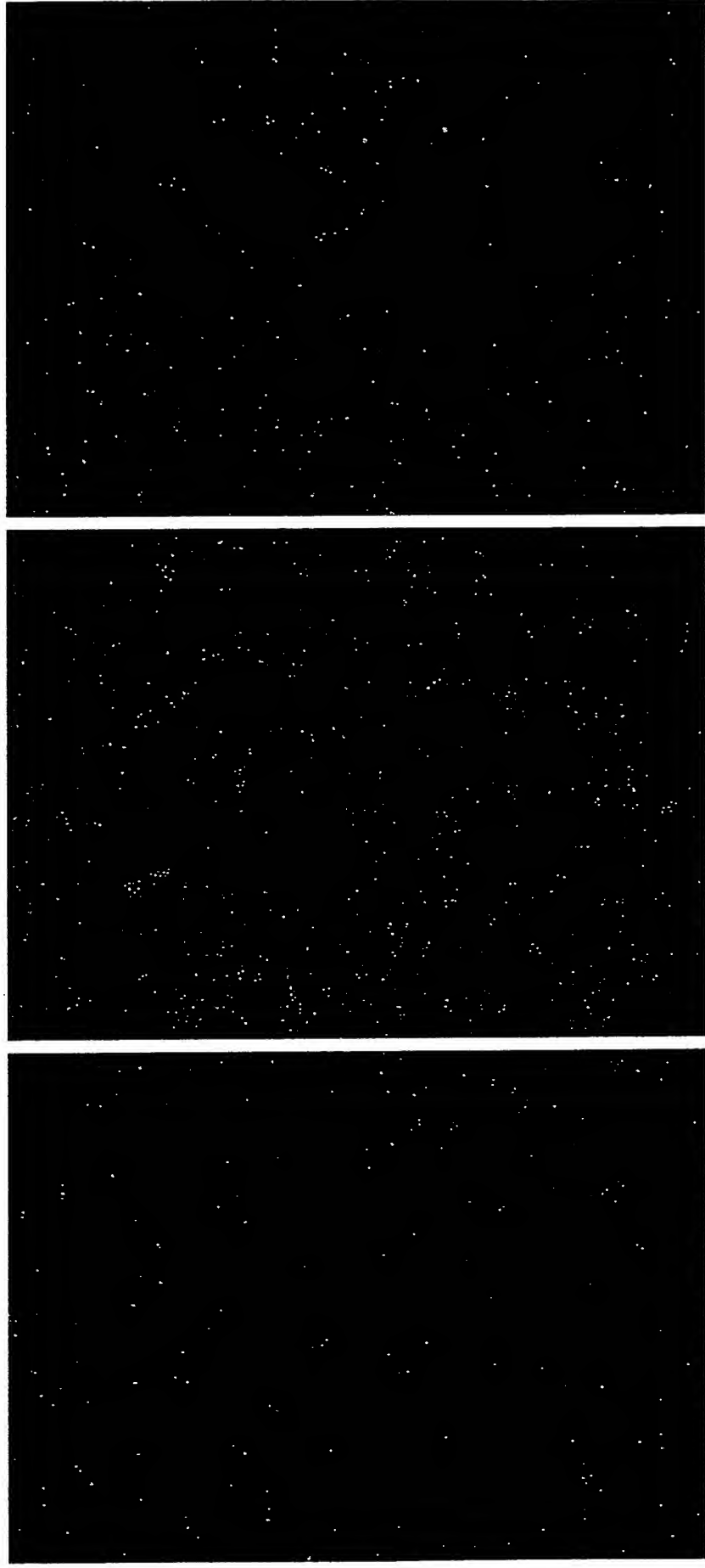
Primary human mesenchymal stem cells

Figure 49



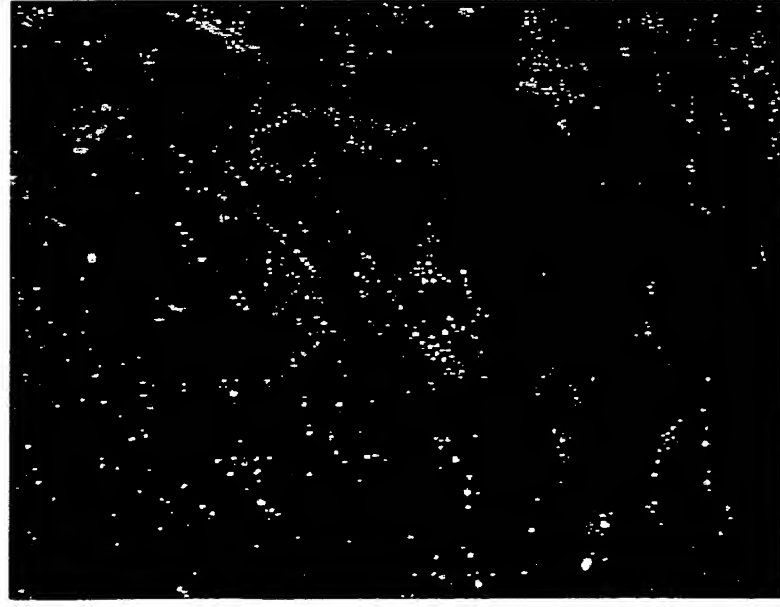
Adipocyte differentiation
Mouse mesenchymal stem cell line
C3H10T1/2

Figure 50



H5-24: adenovirally mediated expression
of CIDEB does not induce any cell death

Figure 51



Nile Red:
Lipid droplets

Hoechst 33342:
nuclei

FIGURE 52

H5-1 DNA sequence (SEQ ID NO:12)

```
1  GCCCACGCGT CCGGTTTTCT ACTTTGCCAC AGATTATCTT GTACAGCCTT TTATGGACCA
61  ATTAGCATTC CATCAATTTT ATATCTAGCA TATTTGCGGT TAGAATCCCA TGGATGTTTC
121 TTCTTTGACT ATAACAAAT CTGGGGAGGA CAAAGGTGAT TTCCTGTGT CCACATCTAA
181 CAAAGTCAAG ATTCCCGGCT GGACTTTTGC AGCTTCCTTC CAAGTCTTCC TGACCACCTT
241 GCACTATTGG ACTTTGGAAG GAGGTGCCTA TAGAAAACGA TTTTGAACAT ACTTCATCGC
301 AGTGGACTGT GTCCCTCGGT GCAGAACTA CCAGATTTGA GGGACGAGGT CAAGGAGATA
361 TGATAGGCCC GGAAGTTGCT GTGCCCCATC AGCAGCTTGA CGCGTGGTCA CAGGACGATT
421 TCACTGACAC TGCGAACCTC CAGGACTACC GTTACCAAGA GGTTAGGTGA AGTGGTTTAA
481 ACCAAACGGA ACTCTTCATC TTAAACTACA CGTTGAAAAT CAACCCAATA ATTCTGTATT
541 AACTGAATTC TGAACCTTTC AGGAGGTACT GTGAGGAAGA GCAGGCACCA GCAGCAGAAT
601 GGGGAATGGA GAGGTGGGCA GGGGTTCAG CTTCCCTTTG ATTTTTTGCT GCAGACTCAT
661 CCTTTTTTAA TGAGACTTGT TTTCCCCTCT CTTTGAGTCA AGTCAAATAT GTAGATTGCC
721 TTTGGCAATT CTTCTTCTCA AGCACTGACA CTCATTACCG TCTGTGATTG CCATTCTTTC
781 CCAAGGCCAG TCTGAACCTG AGGTTGCTTT ATCCTAAAAG TTTTAACCTC AGGTTCCAAA
841 TTCAGTAAAT TTTGGAAACA GTACAGCTAT TTCTCATCAA TTCTCTATCA TGTGAAGTC
901 AAATTTGGAT TTTCCACCAA ATTCTGAATT TGTAGACATA CTTGTACGCT CACTTGCCCC
961 AGATGCCTCC TCTGTCCTCA TTCTTCTCTC CCACACAAGC AGTCTTTTTT TACAGCCAGT
1021 AAGGCAGCTC TGTCGTGGTA GCAGATGGTC CCATTATTCT AGGGTCTTAC TCTTTGTATG
1081 ATGAAAAGAA TGTGTTATGA ATCGGTGCTG TCAGCCCTGC TGTCAGACCT TCTTCCACAG
1141 CAAATGAGAT GTATGCCCAA AGACGGTAGA ATTAAAGAAG AGTAAAATGG CTGTTGAAGC
1201 AAAAAAAAAA AAAAA
```

FIGURE 53

H5-24 DNA sequence (SEQ ID NO:14)

```

1  GTCGACCCAC GCGTCCGCGC CTGCAGAAGG TTGACTGCGT GGTAGGGGGC CCAGAGCAAG
61  CCGAAGGCCAA GCACGATGGC GCTCACCAGC CGGCCACCC GCGCCCCGTG CCGCCCGGAG
121 CCCCAGCGGG CGCCCCGAG CCGTGCCAGC GTCACGCTGT AGCAGCCGAG CATCAGCCCCG
181 AAAGGAAGCA CGAAAGCGGT GGCGGTAGAC GGCGGCCGGG ACGGCGAGCA ACAGGGCGGC
241 CAGCCAGACC GCCAGCAGCA GGCGGCCGGG CAGGGCCGGG CTGCGCAGCC GAGGCGCCAG
301 GAAGGGGCGG GTGACTGCGA GGCAGCGCTG CAGGCTGAGC AGGCCGGTGA GCAGCACGCT
361 GGCCTACATG CTGAGCGCGC ACACGTAGTA CACCGCCTTG CAGCCCGCCT GGCCCGAGCG
421 CCAGGCCTGC CGGGTCAGGA AGGCCACAAA GAGCGGCGTG AGCAGCAGCA CCGCGCCGTG
481 GGCCAGCGCC AGGTGCAGCA CAAGCGTGGC CGCCAGCGGT CGCCCCCGTG CAGGCCGCCA
541 GCCCGCCAAG CTCCACACCA CGAAGCCGTT GCCAGGCAGC CCCAGCAGCG CCGCCAGCAG
601 CAGGAAGGCT GTGCCTGTGG CCCGCGAAGT CTTCCAGTCT AGCAGTGTCT CGTTCCCTGG
661 GGGACGGTAG CAGACCGACA TCCTTCTGGG CCTACAGGAC ACAGAAAAAA AGTGGGGAAG
721 CTGGGGGACC CCTACAAGGA TCCTTGCCAG GAAAGCAGGG ATTGTGTTCA TTTGAGGGTT
781 TCACTGTCTG TGAGAGTCTC AGCTTCCATG CAACTGTCCA TCACGGCTGC AACTGAAATC
841 AGAGCTGGGA CACAGCGCAC CAGAAGCTAA AGTCTTGATG CCATCAAAGG ACATCCCTGC
901 CCCATTCACT TCTCTGTCTC GTCCACTAAT CGGCAAAAGG AGAAAAGTGA GAGAAGATGA
961 CCTAAGTGTG ACTGCAGCAG GCAGCTCTGG AAAATGAAGC CAGAGCAGTG AGCCAGCCCC
1021 TCCTCCGACC AAGGAGGAAG GAAAGAGCAG CCCCAGCACA GGAGAGAACC ACCCAGCCCA
1081 GAAGTTCCAG GGAAGGAACT CTCCGGTCCA CCATGGAGTA CCTCTCAGCT CTGAACCCCA
1141 GTGACTTACT CAGGTCAGTA TCTAATATAA GCTCGGAGTT TGGACGGAGG GTCTGGACCT
1201 CAGCTCCACC ACCCCAGCGA CCTTCCGTG TCTGTGATCA CAAGCGGACC ATCCGGAAAG
1261 GCCTGACAGC TGCCACCCGC CAGGAGCTGC TAGCCAAAGC ATTGGAGACC CTACTGCTGA
1321 ATGGAGTGCT AACCCTGGTG CTAGAGGAGG ATGGAAGTGC AGTGGACAGT GAGGACTTCT
1381 TCCAGTGCTG GGAGGATGAC ACGTGCCTGA TGGTGTGCA GTCTGGTCAG AGCTGGAGCC
1441 CTACAAGGAG TGGAGTGCTG TCATATGGCC TGGGACGGGA GAGGCCAAG CACAGCAAGG
1501 ACATCGCCCG ATTACCTTT GACGTGTACA AGCAAAACCC TCGAGACCTC TTTGGCAGCC
1561 TGAATGTCAA AGCCACATTC TACGGGCTCT ACTCTATGAG TTGTGACTTT CAAGGACTTG
1621 GCCCAAAGAA AGTACTCAGG GAGCTCCTTC GTTGGACCTC CACTGCTG CAAAGCCTGG
1681 GCCATATGTT GCTGGGAATT TCCTCCACCC TTCGTATG CAGTGGAGGG GCTGAGCAGT
1741 GGCAGCAGAA GGGCCGCCTC CATTCCTACT AAGGGGCTCT GAGCTTCTGC CCCAGAATC
1801 ATTCCAACCG ACCCACTGCA AAGACTATGA CAGCATCAA TTTCAGGACC TGCAGACAGT
1861 ACAGGCTAGA TAACCCACCC AATTTCCTCT CTGTCTCTG ATCCCTCGT GACAGAACCT
1921 TTCAGCATAA CGCTCACAT CCCAAGTCTA TACCCTTACC TGAAGAATGC TGTCTTTCC
1981 TAGCCACCTT TTAGCCTCC CACTTGCCCT GAAAGGCCAA GATCAAGATG TCCCCAGGC
2041 ATCTTGATCC CAGCCTGACT GCTGCTACAT CTAATCCCCT ACCAATGCCT CCTGTCCCTA
2101 AACTCCCCAG CATACTGATG ACAGCCCTCT CTGACTTTAC CTTGAGATCT GTCTTCATAC
2161 CCTTCCCCTC AACTAACAA AAACATTTCC AATAAAAAATA TCAAATATTT AAAAAAAAAA
2221 AAAAAAAGGG CGGCCGC

```

FIGURE 54

H5-24 ORF4 Amino Acid sequence (SEQ ID NO: 71)

MEYLSALNPSDLLRSVSNISSEFGRRVWTSAPPPQRPFRVCDHKRTIRKGLTAAT
RQELLAKALETLLNGVLTLVLEEDGTAVDSEDDFFQLLEDDTCLMVLQSGQSWS
PTRSGVLSYGLGRERPKHSDIARFTFDVYKQNPRDLFGSLNVKATFYGLYSMS
CDFQGLGPKKVLRELLRWTSTLLQGLGHMLLGISSTLRHAVEGAEQWQQKGRL
HSY

FIGURE 55

H5-24 Segment 1 of BLTR2 DNA sequence (SEQ ID NO: 15)

```
      18 CGC CTGCAGAAGG TTGACTGCGT GGTAGGGGGC CCAGAGCAAG
61  CCGAAGGCAA GCACGATGGC GCTCACCAGC CGGCCCCACCC GCGCCCCGTG CCGCCCCGAG
121 CCCCAGCGGG CGCCCCGCAG CCGTGCCAGC GTCACGCTGT AGCAGCCGAG CATCAGCCCC
181 AAAGGAAGCA CGAAAGCGGT 200
```

FIGURE 56

H5-24 Segment 2 DNA sequence (SEQ ID NO: 16)

```
      198 GGT GGC GGCTAGAC GCGGCCGGG ACGGCGAGCA ACAGGGCGGC
241 CAGCCAGACC GCCAGCAGCA GCGGGCGGGC CAGGGCCGGG CTGCGCAGCC GAGGCGCCAG
301 GAAGGGGCGG GTGACTGCGA GGCAGCGCTG CAGGCTGAGC AGGCCGGTGA GCAGCACGCT
361 GCGGTACATG CTGAGCGCGC ACACGTAGTA CACCGCCTTG CAGCCCGCCT GGCCAGCGG
421 CCAGGCCTGC CGGGTCAGGA AGGCCACAAA GAGCGGCGTG AGCAGCAGCA CCGCGCCGTC
481 GGCCAGCGCC AGGTGCAGCA CAAGCGTGGC CGCCAGCGGT CGCCCCCGTG CAGGCCGCCA
541 GCCCGCCAAG CTCCACACCA CGAAGCCGTT GCCAGGCAGC CCCAGCAGCG CCGCCAGCAG
601 CAGGAAGGCT GTGCCTGTGG CCCGCGAAGT CTTCCAGCTC AGCAGTGTCT CGTTCCTTGG
661 GGGACGGTAG CAGACCGACA TCCTTCTGGG CCTACAGG 698
```

FIGURE 57

DNA Sequence Comparison of H5-24 Segment 1 (SEQ ID NO: 15) with BLTR2

Antisense DNA sequence

```

SEQ ID NO:15   18  cgctgcagaaggttgactgcgtggtagggggccagagcaagccgaaggcaagcacgat  77
                  |||
BLTR2          2455 cgctgcagaaggttgactgcgtggtagggggccagagcaagccgaaggcaagcacgat  2396

SEQ ID NO:15   78  ggcgctcaccagccggccaccgcgccccgtgccgcccggagccccagcggcgccccg  137
                  |||
BLTR2          2395 ggcgctcaccagccggccaccgcgccccgtgccgcccggagccccagcggcgccccg  2336

SEQ ID NO:15   138  cagccgtgccagcgtcacgctgtagcagccgagcatcagcccgaaggaagcacgaaagc  197
                  |||
BLTR2          2335 cagccgtgccagcgtcacgctgtagcagccgagcatcagcccgaaggaagcacgaaagc  2276

SEQ ID NO:15   198  ggt  200
                  |||
BLTR2          2275 ggt  2273

```

